ANALYSIS OF THE ACCUMULATION, TOXIC EFFECTS, AND RISK OF PERSISTENT ORGANIC POLLUTANTS IN PINNIPEDS

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The present studies determine the accumulation of persistent organic pollutants (POPs) in three pinniped species, evaluate the relationship with relevant biomarkers of exposure, and calculate toxic effect thresholds. Stranded harp and hooded seals were found to be accumulating PBDEs at levels which could pose a based on threshold levels determined in this study. Northern fur seals are accumulating all three classes of POPs (PCBs, PBDEs, and OCPs) with significant relationships being seen with blubber percent lipid. Correlations between contaminant concentrations and expression levels of relevant biomarkers were seen potentially indicating an effect on multiple pathways. Overall risk can be hard to determine due to factors such as sex and age. Broad threshold response values and hazard quotients were calculated for toxic effect endpoints in pinnipeds. Overall these results suggest that certain populations of pinnipeds are at high risk of experiencing toxic effects due to POP exposure, but it is important to understand effects even at lower concentrations. The relationship between exposure, toxic effects, and other stressors, both environmental and physiological, can impact the overall fitness and survival of pinnipeds.

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CHAPTER 1

INTRODUCTION

Persistent organic pollutants (POPs) are toxic contaminants, often organohalogens, which are hazardous to human and environmental health. A global treaty, the Stockholm Convention, was adopted in 2001 to work towards the elimination of twelve historic or legacy POPs, including polychlorinated biphenyls (PCBs), DDT, and other organochlorine pesticides (OCPs) (Stockholm Convention, 2011; U.S. Environmental Protection Agency, 2011). As more research emerged, the treaty was amended to include nine emerging POPs, including polybrominated diphenyl ethers (PBDEs) (Stockholm Convention, 2011; U.S. Environmental Protection Agency, 2011).

POPs have long environmental half-lives and can undergo long-term atmospheric transport. This allows for them to sustain their persistence once in the environment, especially in cold ecosystems, where they can bioaccumulate up the food chain (Simonich and Hites, 1995; Wania and Dugani, 2003). Globally, POP concentrations have been found at toxicologically relevant levels causing them to be considered ubiquitous environmental contaminants (de Wit and Muir, 2010; Wania and Mackay, 1996). Oceans act as sinks for POPs once they have been released into the atmosphere, due to rapid absorption to organic matter (O'Shea, 1999). Three classes of POPs, two legacy: PCBs and OCPs and one emerging: PBDEs, have been found to accumulate throughout marine food webs due to their persistence and low metabolic degradation (O'Hara and O-Shea, 2001; O'Shea, 1999).

PCBs are produced through the chlorination of biphenyls with the number and location of the chlorines differentiating the 209 congeners. They previously had wide application in

industry being used in everything from electrical transformers and capacitators to plastics and paint (O'Hara and O-Shea, 2001; O'Shea, 1999; Safe and Hutzinger, 1984). PCBs were discovered as an environmental contaminant in the late 1960s with most industrialized nations stopping production in the 1970s and 1980s (Hutchinson and Simmonds, 1994; O'Shea, 1999). A large amount of PCBs that were produced are still contained in the closed systems they were designed for (O'Hara and O'Shea, 2001; O'Shea, 1999). As these systems begin to degrade or are disposed of, the PCBs contained within them will be released into the environment meaning that the amount reaching the marine system will continue to increase.

OCPs encompass a large group of compounds including DDT and its metabolites, hexachlorocyclohexane (HCH), toxaphenes, and cyclodienes, including dieldrin and chlordane. The original “dirty dozen” that lead to the Stockholm Convention is dominated by these compounds and their metabolites/degradation products (Stockholm Convention, 2011; U.S. Environmental Protection Agency, 2011). DDT and its metabolites are the most commonly found organochlorine in tissues of marine organisms (de Wit and Muir, 2010; O'Shea, 1999). The production of DDT greatly increased in the 1940s when its properties as potent insecticide were discovered (Hutchinson and Simmonds, 1994). Much like PCBs, the environmental impact of them was not discovered until decades later when DDT became the contaminant of the movement to regulate contaminants. The effectiveness of DDT led to the creation of many of the other OCPs, that were in turn also found to be toxic environmental contaminants (Hutchinson and Simmonds, 1994). A major concern in understanding the toxicity of OCPs is the wide variety of degradation products that often have different properties than the parent compounds including toxicity level and persistence.

PBDEs are produced by the bromination of a diphenyl ether in the presence of a catalyst (Rahman et al., 2001) with the location and number of bromine ions determining the 209 congeners. The presence of an ether linkage influences the persistence and partitioning of PBDEs in the environment (Ross, 2006). PBDEs were widely used flame-retardants on everything from electronics and building materials to textile products (Betts, 2008; Shaw and Kannan, 2009). Temporal studies examining environmental concentrations of PBDEs led to the banning of the penta- and octa-BDE forms in the U.S. and Europe in the early 2000s (Alaee et al., 2003; Shaw et al., 2008). In 2009, tetra- and hepta-BDEs were listed under the Stockholm Convention (McKinney et al., 2011; Stockholm Convention, 2011; U.S. Environmental Protection Agency, 2011).

1.1 PCBs, OCPs, and PBDEs in the Environment

PCBs, OCPs, and PBDEs share similar chemical properties that allow them to persist in the environment and enter the food chain. They all tend to have low reactivity, are volatile at normal temperatures allowing for atmospheric transport, and are highly lipophilic (de Wit, 2002; Hutchinson and Simmonds, 1994). All of these compounds often enter the environment through leaking, leaching of compounds, and during disposal. Once in the environment, they can enter marine environments through terrestrial runoff or more often through atmospheric deposition after transport on particulate matter (Braune et al., 2005; de Wit et al., 2006; O'Hara and O'Shea, 2001). In the marine environment, there tends to be a gradient of increasing concentrations from the tropics to the poles for most organohalogens (Jenssen et al., 2007).

After entering the marine environment, these compounds easily enter the food web through binding to sediment and other suspended particles (Braune et al., 2005) becoming

bioavailable to higher trophic levels. Levels of these compounds in Arctic sediments range from <0.01-0.57 ng/g dw for PCBS and OCPs (Kelly et al., 2007) and from 0.1-1.6 ng/g dw for PBDEs (Kelly et al., 2008a; Kelly et al., 2008b). These compounds have the ability to bioaccumulate and biomagnify as they move up the food web due to the strong affinity for lipids and biological inertness of many of the parent compounds and metabolites (Braune et al., 2005; de Wit, 2002). Bioconcentration is highest at the first trophic level, with the difference in concentration becoming higher with each step up the food web due to low rates of metabolism and excretion (Braune et al., 2005).

For animals in the marine environment, the main route of exposure is diet with maternal transfer being important in higher trophic level species like marine mammals (Braune et al., 2005; Shaw and Kannan, 2009). Invertebrates and fish species can be good indicators of which environmental contaminants are present in a given system since they are accumulating POPs from the water, sediments, and prey species (Brown et al., 2018). Concentrations in marine invertebrates, such as copepods, vary greatly by location and the diet of the species (Braune et al., 2005; Brown et al., 2018; Kelly et al., 2008a). For some species, more water-soluble compounds are found in higher concentrations whereas for some zooplankton lipophilic compounds have the highest levels (Brown et al., 2018; Muir et al., 2013; Muir, 2003). In marine fish, concentrations of PCBs, OCPs, and PBDEs can vary by prey species and trophic position. High levels of PCBs have been found in salmon (129 ng/g lw) and Greenland sharks (2000 ng/g ww) (Fisk et al., 2002; Kelly et al., 2007). In a common Arctic prey species, Arctic cod, PCBs are found in the highest concentrations with DDT and HCH occurring at lower levels (Kelly et al., 2007). PBDE concentrations in Arctic cod range from 9.8-23 ng/g lw across the

Arctic (Kelly et al., 2008a; Tomy et al., 2008). Trophic magnification factors for these compounds vary anywhere from 0.76 for some PBDEs up to over 10 for certain PCB congeners and DDT metabolites (Kelly et al., 2008a; Muir et al., 2013). This means that as POPs are moved up the food web, higher and higher concentrations of PBCs, OCPs, and PBDEs will be seen in species like marine mammals.

1.2 PCBs, OCPs, and PBDEs in Marine Mammals

Increased levels of POPs in Arctic and Antarctic biota can indicate a growing environmental problem (de Wit et al., 2006; Ikonomou et al., 2002). Arctic and Antarctic systems are important systems for all levels of marine life, serving as areas of high production. With increasing levels of persistent, lipophilic compounds, such as PCBs, OCPs, and PBDEs, in marine systems and particularly polar regions it is important to understand the impact on large, apex predators like marine mammals.

Marine mammals are sentinel species for the systems they inhabit (Cipro et al., 2012). Many species have seasonal migrations and changes in diet, which can cause variation in exposure to toxicants (Cipro et al., 2012). The life history strategies, long life, and high trophic position make marine mammals vulnerable to biomagnification of toxicants (Desforges et al., 2013; Fair et al., 2010). Marine mammals feed on a wide variety of prey species and in a variety of habitats. Different feeding habits can lead to differences in POP contamination (Muir et al., 2000). The location of foraging can affect POP contamination, especially for species that inhabit areas with direct pollution inputs (Blasius and Goodmanlowe, 2008; Brown et al., 2014a).

Marine mammal species can be particularly susceptible to toxicant exposure due to their dependence on blubber as a source of energy during multiple life history stages (Wolkers

et al., 2006). Species inhabiting Arctic and sub-Arctic regions, such as seals, can have upwards of 30% of their body mass composed of blubber with >90% of total body lipid being in the blubber (Tanabe et al., 1981). In some species of marine mammals, the blubber contains 90-95% of the organohalogen accumulation (Tanabe et al., 1981). When those blubber stores are mobilized for energy, they are simultaneously releasing stored toxicants into circulation causing them to be more bioavailable (Ikonomou and Addison, 2008; Wolkers et al., 2006) or concentrating contaminants in the remaining blubber (O'Hara and O'Shea, 2001).

Life history and demographic factors, such as age, sex, and reproductive status, can also be sources of variation in POP accumulation. Young, immature animals usually have similar concentrations when comparing between the sexes (O'Hara and O'Shea, 2001; O'Shea, 1999). When comparing between mature males and females though, significant differences can be seen between the sexes with males most often having higher body burdens of POPs (Borrell, 1993; O'Hara and O'Shea, 2001; O'Shea, 1999). The differences seen in male and female burdens is likely due to maternal transfer of POPs mostly through the production of lipid-rich milk. Studies have shown that upwards of 90% of the body burden of some compounds in young animals at weaning is obtained through milk (Addison and Stobo, 1993; Bacon et al., 1992; Frouin et al., 2012). The highest levels of maternal transfer are seen in pinnipeds and odontocetes, with baleen whales transferring lower proportions (Borrell, 1993; O'Hara and O'Shea, 2001). These high levels of transfer from mother to young can potentially have ramifications for the health of the young (Beckmen et al., 1999).

1.3 Pinnipeds

Pinnipeds represent just over a quarter of the total marine mammals found globally.

They are large, long-lived species with delayed sexual maturity that inhabit almost all aquatic environments including many in polar regions (Berta, 2017; Berta et al., 2005). Pinnipeds exhibit a wide range of breeding strategies from polygyny to single mating and have different lactation strategies often based on species and pupping locations pairs (Berta, 2017; Riedman, 1990). The species of pinnipeds are broken into two main groups (walruses are their own group of pinniped): phocids, true, or earless seals that represent over 50% of the species, and otariids, eared seals (fur seals and sea lions) that represent the remaining species.

Phocid seals spend a large part of their life at sea returning to land or ice for pupping, breeding, and molting. Most species of phocid will forage for long periods before giving birth and then fast while lactating because feeding sites are far from breeding sites (Riedman, 1990). The exception to this is harbor seals which will take short feeding bouts while nursing similar to otariids (Berta, 2017; Riedman, 1990). Phocids produce a fat-rich milk allowing for pups to get a large amount of energy in a rather short time period (Riedman, 1990). Hooded seals wean their pups in less than five days with milk that can be up to 90% lipid where as more temperate species like monk seals wean pups in 5-7 weeks (Lavigne and Kovacs, 1998; Riedman, 1990). After weaning, pups are reliant on their fat stores for weeks or months before being able to forage on their own.

Otariids are distinguished from phocids by their external ears as well as having a semi-aquatic lifestyle. They are further divided into fur seals and sea lions with the major distinction being the thicker underfur layer found in fur seals (Riedman, 1990). Fur seals are mostly found in the southern hemisphere (with the northern fur seal being the exception). They are also generally smaller, more sexually dimorphic, and go on longer foraging trips than sea lions

(Berta, 2017; Berta et al., 2005; Riedman, 1990). Otariids form annual breeding aggregations on beaches and rocky coastlines with all species being polygynous (Berta, 2017; Riedman, 1990). Female otariids will nurse pups for a few days at a time then forage for a few days/weeks while leaving the pup onshore (Berta et al., 2005). The pups will nurse anywhere from 6-11 months with a few species nursing for over a year (Berta, 2017; Berta et al., 2005). At weaning, otariid pups are usually smaller and weigh less than phocid pups, but may enter the water sooner and learn to swim at an earlier age (Berta, 2017; Berta et al., 2005).

1.4 Accumulation of PCBs, OCPs, and PBDEs in Pinnipeds

Pinnipeds were some of the first marine organisms to have PCBs and other POPs detected in their tissues (Jensen, 1966). Since the discovery of persistent contaminants, concentrations of PCBs, OCPs, and PBDEs have been reported in thousands of samples from pinnipeds across the globe. Most studies looking at accumulation have focused on harbor, ringed, and grey seals likely due to ease of sampling and having large populations across the northern hemisphere.

PCB profiles in pinniped tissue tend to be dominated by hexa- and hepta-CBs. In harbor seals and ringed seals, ΣPCB levels range from 78-44,000 ng/g and 190-64,500 ng/g, respectively. The highest levels of ΣPCB in harbor seals were seen in the blubber and liver of adults sampled in California (Blasius and Goodmanlowe, 2008; Kajiwara et al., 2001). In ringed seals, the highest PCB levels are found in seals sampled in the Baltic, which were some of the original seals studied for PCB contamination (Jensen, 1966). Some of the lowest PCB levels have been seen in harp seals (Frouin et al., 2012)and ribbon seals (Chiba et al., 2001; Nomiyama et al., 2014). A study done looking at maternal transfer of PCBs in hooded seals showed that ΣPCB

levels in pups represented about 35% of that seen in females with the lower chlorinated compounds being more readily transferred during lactation (Wolkers et al., 2006).

A large number of OCPs have been measured in pinniped tissues with DDT and its metabolites being the most common. California sea lions and harbor seals sampled in California have some of the highest levels of DDT, DDD, and DDE with ΣDDT concentrations >35,000 ng/g (Blasius and Goodmanlowe, 2008; Kajiwara et al., 2001). Spotted seals, sampled in the Sea of Japan, had high ΣDDT levels ranging from 17,000-400,000 ng/g (Trukhin and Boyarova, 2013; Trukhin and Boyarova, 2020). In all three species, DDE represented the highest measured compound, which is common across pinniped species. When looking at other OCPs, chlordane's and HCHs are often found in pinniped tissue sometimes exceeding 1,000 ng/g (Kajiwara et al., 2001; Trukhin and Boyarova, 2013; Trukhin and Boyarova, 2020). Wolkers et al. (Wolkers et al., 2006) found, when looking at maternal transfer that the pup burden of DDE, toxaphenes, and chlordane's was 50% that of what was found in the mother.

The most common congener of PBDEs seen in seal species is BDE-47 and comprised the greatest percentage of total PBDEs (Frouin et al., 2011; Ikonomou and Addison, 2008; Shaw et al., 2012; Shaw et al., 2008). Studies have shown that total PBDEs in harbor seals can range from 1,000-4,000 ng/g (Shaw et al., 2012; Shaw et al., 2008). Frouin et al. (Frouin et al., 2011) looked at PBDE concentrations in harbor, grey and harp seal pups with harbor seals having the highest accumulation rates (530 ng\g) and harp seals having the lowest (21 ng\g). Ikonomou et al. (Ikonomou and Addison, 2008) showed that transfer efficiency from mom to pup in grey seals increased with decreasing levels of bromination with upwards of 44% of ΣPBDEs transferred to the pup. Similar studies done in harbor seals showed the same patterns of

transfer from mom to pup with pups and yearlings exhibiting the highest levels (Shaw et al., 2008).

1.5 Toxicity of PCBs, OCPs, and PBDEs in Pinnipeds

Most POPs are effectively taken up across the gastrointestinal tract where they can be deposited in blubber or liver tissue. All three compound classes, PCBs, OCPs, and PBDEs, have been shown to be immunotoxic, hepatotoxic, endocrine disrupters, and potentially impact reproduction. The mixed function oxidase (MFO) system is the main enzyme pathway for the metabolism of contaminants with hundreds of contaminants known to cause enzyme induction (Boon et al., 1994; Boon et al., 1992; O'Hara and O'Shea, 2001; O'Shea, 1999).

Organohalogen compounds are lipophilic and need to be made into more polar products in order to be excreted (Boon et al., 1994; Boon et al., 1992), but for some compounds, the produced metabolite is more toxic than the parent compound (Boon et al., 1992; O'Hara and O'Shea, 2001; O'Shea, 1999). The cytochrome P-450 (CYP450) enzymes are part of the MFO system and bind the contaminant with oxygen to increase the polarity. Different organohalogen compounds can act as CYP450 enzyme inducers, inhibitors, or substrates. In marine mammal tissue, multiple compounds and enzymes are present meaning that the system can oxidize many different compounds at once with the ability to metabolize organohalogens increasing with increasing exposure (O'Hara and O'Shea, 2001; O'Shea, 1999). Increased MFO activity at increasing rates of contamination can help to explain why some congeners are not seen in tissues or why congeners in tissue is not always reflective of the technical mixtures produced. The induction of CYP450 enzymes can also potentially result in altered metabolism rates of endogenous compounds, like hormones, which can in turn affect

developmental and physiological processes (Boon et al., 1992; O'Hara and O'Shea, 2001).

Studies suggesting a link between PCB and DDT exposure in pinnipeds and impacts on reproduction were first conducted in the 1970s. A number of studies saw that ringed seals in the Baltic Sea with high levels of DDTs and PCBs had higher occurrences of uterine occlusions and stenosis (Helle, 1976; Helle et al., 1976). DeLong et al. (DeLong et al., 1973) found that California sea lions with high contaminant loads had higher abortion and stillbirth rates. Later studies looking at reproduction did not find correlation between PCBs and changes in uterine pathology (Gun et al., 1992) suggesting that the previous findings confounded due to other factors such as age or disease (O'Hara and O'Shea, 2001). A study done on captive harbor seals showed that decreased reproductive success, due to PCB and OCP exposure, was likely failure at the implantation stage (Reijnders, 1986).

Endocrine-related impacts on hormones related to growth, development, and reproduction have been seen in pinniped species exposed to organohalogen compounds. A decrease in reproductive hormones, such as estradiol and progesterone, has been observed with increasing levels of PCBs (Troisi et al., 2020), while DDT and DDE can potentially disrupt estrogen receptor signaling pathways (Yoshinouchi et al., 2019). Contaminant related effects on thyroid hormones have been shown for PCBs, OCPs, and PBDEs in pinnipeds. Thyroxine (T_4) is the most highly produced hormone and crucial in the production of the more bioactive triiodothyronine (T_3) through deiodinase activity (Tabuchi et al., 2006). Both thyroid hormones have an effect on other hormones and enzymes mostly related to metabolism, growth, and development (Hall et al., 1998; Hall et al., 2003). Both thyroid hormones have shown to be impacted by all three classes of compounds in both free and total forms as well as the

relationships between T_4 and T_3 (Gronnestad et al., 2018; Hall and Thomas, 2007; Sormo et al., 2005; Villanger et al., 2013). There is also evidence that hydroxylated metabolites may interact with thyroid hormone receptors. Interaction with nuclear receptors can alter transcription activity and gene expression influencing regulation of genes that are crucial at different developmental stages (de Wit, 2002; Tabuchi et al., 2006).

Contaminant-induced immunosuppression has been observed in laboratory specimens and is a growing concern with pinnipeds due to the potential for increased susceptibility to disease. A cause and effect linkage is difficult due to other factors, such as population density, habitat disturbance, and climate changes, which can influence the introduction and spread of disease (Kannan et al., 2000; Lahvis et al., 1995). A series of studies done on semi-captive harbor seals demonstrated decreased immune function in seals fed contaminated fish: low white blood cell counts, lower serum vitamin A, decreased T and B lymphocyte response, and lower natural killer cell activity (de Swart, 1995; De Swart et al., 1996; Deswart et al., 1995; Ross et al., 1995; Ross et al., 1996). A study on harbor seal pup immune cells showed dose-dependent effects on disruption of the immune system including decreased phagocytosis and increased reactive oxygen species production (Frouin et al., 2010). Overall, humoral immunity was affected less than cellular immunity.

1.6 Study Objectives

Pinnipeds are exposed to POPs through dietary accumulation and maternal transfer during lactation. Most of these compounds are highly lipophilic and are stored in blubber. Pinnipeds utilize blubber as an energy source during periods of fasting that may occur during migrations, lactation, and molting (Berta, 2017; Berta et al., 2005; Riedman, 1990). Seal pups,

especially phocid pups and those occurring in higher latitudes, rely on the blubber stores that they develop during nursing for energy before they are ready to begin foraging on their own (Lavigne and Kovacs, 1998; Riedman, 1990). Being high tropic level predators that are long-lived, pinnipeds are susceptible to have high body burdens of POPs that can potentially affect immune function, endocrine cycling, reproduction, and eventually overall fitness of the species.

The present study quantified concentrations of PCBs, PBDEs, and OCPs in the blubber of three species of Arctic and sub-Arctic seals, determined relationships between contaminant levels and molecular biomarkers of contaminant exposure, and conducted a quantitative risk analysis of PCBs, PBDEs, and OCPs in pinnipeds. The first component of the study determined if PBDEs, an emerging POP, were accumulating in the blubber of stranded harp and hooded seals, animals that can be considered immune compromised, and compare accumulated levels with demographic factors to assess differences between and within species. The second component determined PCB, OCP, and PBDE loads in the blubber of subsistence harvested northern fur seals as well as measured biomarkers of contaminant exposure in blubber. The relationship between contaminant data and changes in gene expression can start to give a sense of how the contaminants are affecting the seals once taken up. The goal of the final component is to conduct a comprehensive, quantitative risk analysis of POPs in pinnipeds sampled over the last 20 years to understand the potential relationship between toxic effects and POP accumulation in wild seals.

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CHAPTER 2

ACCUMULATION OF PBDES IN STRANDED HARP (*Pagophilus groenlandicus*) AND HOODED SEALS (*Cystophora cristata*) FROM THE NORTHEASTERN UNITED STATES1

2.1 Introduction

Flame retardants are substances used in a range of products from textiles to plastics to electrical circuitry to increase fire resistance of materials (Alaee et al., 2003; Shaw et al., 2008). Halogens are especially effective at capturing free radicals during the combustion process making organohalogen compounds effective flame retardants (Alaee et al., 2003). Polybrominated diphenyl ethers (PBDEs) are a class of brominated flame retardants (BFRs) that have been widely used in a variety of consumer products since the 1960s. Within the global market, there are three commercial mixtures of PBDEs; penta-, octa-, and deca-BDE. Penta- and octa-BDE forms were banned in the U.S. and Europe in the early 2000s due to environmental health concerns (Betts, 2002; de Wit, 2002; de Wit et al., 2006). Prior to the ban in the U.S., use of PBDE based compounds doubled every 2-5 years (Betts, 2002).

PBDEs are highly lipophilic compounds that can persist in the environment and have the potential to bioaccumulate (de Wit, 2002; Ikonomou and Addison, 2008). The presence of an ether linkage influences the persistence and partitioning of PBDEs in the environment (Ross, 2006). The chemical nature of PBDEs (log K_{OW} ranging from 5.9-10) allows them to leach from surfaces and enter the environment as well as having a high binding affinity for particulates (de

1 This chapter is presented in its entirety from Soulen, BK, Venables BJ, Johnston DW, Roberts AP. Accumulation of PBDEs in stranded harp (Pagophilus groenlandicus) and hooded seals (Cystophora cristata) from the Northeastern United States. Marine Environmental Research 2018; 138: 96-101 with permission from Elsevier.

Wit, 2002; Shaw et al., 2008). Once in the environment, PBDEs are mostly resistant to degradation, but debromination from heavy to lighter compounds can occur (Darnerud et al., 2001; de Wit, 2002; Rahman et al., 2001; Ross, 2006). Due to their persistence and widespread use, PBDEs are a ubiquitous environmental contaminant even in remote areas (de Wit et al., 2006; Mongillo et al., 2012).

After entering the environment, PBDEs can travel easily through the atmosphere, with low molecular weight (debrominated) congeners travelling to high latitude areas, before being deposited into the marine environment (de Wit et al., 2006; Ikonomou et al., 2002; Ross et al., 2009). The presence of lower brominated compounds in sink regions, such as Arctic marine environments, provide evidence for the global fractionation of PBDEs in the environment (Ellisor et al., 2013; Ross, 2006). Similar to other organohalogens in the marine environment, there is a gradient of increasing concentrations from the tropics to the poles (Jenssen et al., 2007). Of the 209 congeners in existence, only about 40 of them are commonly detected in marine systems. BDE-47 is the most dominant form found in the environment despite BDE-99 being the most dominant commercial penta-BDE mixture (Kelly et al., 2008a). (Jenssen et al., 2007). The high binding affinity of PBDEs allows them to readily bind to sediment after deposition from which they can enter the food web. As seen with other persistent organic pollutants (POPs), increased levels of PBDEs in Arctic and Antarctic biota can indicate a growing environmental problem (de Wit et al., 2006; Ikonomou et al., 2002). Arctic and Antarctic systems have high seasonal productivity making them important feeding grounds for all levels of marine life.

For animals in marine systems, the two main routes of exposure are diet and maternal

transfer (Shaw and Kannan, 2009). Concentrations of PBDEs within biota in the marine environment are dependent on foraging location and prey species (Johnson-Restrepo et al., 2005; Ross et al., 2009). PBDEs are effectively taken up from the gastrointestinal tract and accumulate mainly in fat depots and liver tissues (de Wit, 2002). Depending on the congener, PBDEs have been shown to be hepatotoxic, immunotoxic, and act as endocrine disrupters (Hall et al., 2003). Penta-BDEs are more toxic when consumed because they are easily absorbed across membranes compared to deca-BDEs that are readily excreted (Darnerud et al., 2001; Hardy, 2002; Kelly et al., 2008a; Shaw and Kannan, 2009) . In the food web, PBDEs can biomagnify (Boon et al., 2002; de Wit et al., 2006). Biomagnification factors in different marine systems range from 3- 85 depending on the congener (Johnson-Restrepo et al., 2005; Shaw and Kannan, 2009). Studies have shown that biomagnification increases with increasing bromination to the hexa-BDE level (Shaw and Kannan, 2009).

Because these compounds can biomagnify, it is important to understand the impact on large, high trophic-level predators like marine mammals. Marine mammals are particularly vulnerable to toxicants that biomagnify due to their long life and high trophic position (Desforges et al., 2013; Fair et al., 2010). Their dependence on mobilization of blubber as a source of energy adds to the potential for long-term exposure (Wolkers et al., 2006). Species inhabiting Arctic and sub-Arctic regions, such as seals, can have upwards of 30% of their body mass composed of blubber. When those blubber stores are mobilized for energy, stored toxicants are re-released into circulation (Ikonomou and Addison, 2008; Wolkers et al., 2006). Lactational transfer of PBDEs in high fat milk is another exposure route to marine mammals, especially in pinnipeds (Cipro et al., 2012). Pinniped species inhabiting polar and sub-polar

regions, especially phocid seals, are particularly susceptible to accumulating high levels of PBDEs and experiencing high rates of maternal transfer (Law et al., 2005).

Previous studies have shown that PBDEs have been found to accumulate in phocid pinnipeds such as harp seals (*Pagophilus groenlandicus*) and hooded seals (*Cystophora cristata*) (Frouin et al., 2011; Ikonomou and Addison, 2008; Wolkers et al., 2006). Phocid seals produce high fat milk to allow for fast weaning of pups. There is a high transfer efficiency from cow to pup of PBDEs and other contaminants (Frouin et al., 2011). Ikonomou et al. (2008) reported that transfer of PBDEs from cow to pup in grey seals increased with decreasing levels of bromination with >44% of ΣPBDEs transferred to the pup. Similar studies done in harbor seals showed the same patterns of transfer from mom to pup with pups and yearlings exhibiting the highest levels (Shaw et al., 2008). Pups are at a crucial stage of growth and development and can be sensitive to even low concentrations of contaminants (Ikonomou and Addison, 2008; Wolkers et al., 2006).

Harp seals and hooded seals are pagophilic, or ice-loving species, that rely on seasonal ice cover to give birth and wean their pups (Lavigne and Kovacs, 1998). These seals breed in the northwest Atlantic off the eastern coast of Canada (Lavigne and Kovacs, 1998; Riedman, 1990). Both of these species are capital breeders that mostly fast during lactation periods relying heavily on blubber stores, which in hooded seals can contain up to 90% lipid (Lavigne and Kovacs, 1998; Law et al., 2005). Pups are normally born from February until March and are weaned within two weeks for harp seals and less than 5 days in hooded seals whose pups are born with a thin layer of blubber (Lavigne and Kovacs, 1998; Riedman, 1990). Because these species wean their pups in such a short time frame, the milk produced is very high in fat

(Riedman, 1990). This high fat milk can transfer contaminants such as PBDEs from mother to pup when fat stores are solubilized.

After weaning, seal pups are highly dependent on a thin layer of blubber and an ice-based food web while learning to forage freely before migrating to feeding grounds (Lavigne and Kovacs, 1998; Simmonds and Isaac, 2007). If this blubber layer has to be mobilized for energy (e.g. changes in ice cover), then contaminates stored in the blubber may be also be mobilized and body condition may decrease (Lehnert et al., 2014). Poor body condition and increased exposure to contaminants like PBDEs, could cause immune suppression and physiological stress in young animals that are still developing (Ikonomou and Addison, 2008; Wolkers et al., 2006). These changes in body condition of yearling (young-of-the-year) animals along with shifts in ice cover can lead to a potential increase in yearling strandings (Johnston et al., 2012; Soulen et al., 2013). Stranded seals represent a group of animals that are immune compromised but little research has been done looking at PBDE loads in these animals. For harp seals, yearling animals are the most likely to strand yearly with increases in strandings being seen in years with light ice cover (Soulen et al., 2013). The goal of this study was to determine concentrations of PBDEs in the blubber of stranded harp and hooded seals and to compare PBDE concentrations against available demographic data.

2.2 Material and Methods

2.2.1 Sample Collection and Stranding Data

Archived blubber samples, stored at -80°C, that were collected from harp and hooded seals that had stranded along the coast of Massachusetts, U.S.A. from 2000-2010 (Table 2.1) were used. Sub-samples of 200-300 mg were taken from each sample for analytical analysis.

Demographics data (e.g. sex, age class, lengths) on stranded seals were obtained from Level A data collected from stranding records (Data available through NOAA Fisheries Office of Protected Resources – see http://www.nmfs.noaa.gov/pr/health/).

Table 2.1: Demographics of stranded seals analyzed for PBDEs. All individuals were sampled from 2000-2010.

Species	Sex	Age Class	Mean Length (cm)	Condition Codes
Harp (n=21)	M: 9 F: 8 U: 4	Yearling: 18 Adult: 3	109.8 ± 20.97	1: 9 2: 2 3: 7 6: 1
Hooded (n=9)	M: 4 F: 5	Yearling:9	93.17 ± 35.75	1: 5 3: 4

2.2.2 Sample Extraction and Clean-up

A sub-sample of blubber (~350 mg) was weighed and placed into a mortar with ~4-5 g of sodium sulfate and ground with a pestle until well homogenized. The homogenized sample was then transferred to a 50 mL Falcon tube and 25 mL of a 50:50 by volume mix of hexane:dichloromethane (DCM, Acros Organics, Pittsburgh, PA) was added. The sample was then vortexed on high for 1 minute and centrifuged at 3,500 rpm for 5 minutes. The resulting extract was placed into a clean 50 mL Falcon tube and the process was repeated with another 25 mL of solvent for a final extract volume of ~50 mL. Two fluorinated-PBDEs (250 ng/g FBDE-47 and 750 ng/g BDE-154; Accustandard, New Haven, CT) were added as internal standards to the extract (Liu et al., 2006; Luthe et al., 2006). The extract was evaporated to ~5 mL under nitrogen in a water bath. The extract was then filtered through a 0.45 µM glass microfiber syringeless filter (Whatman, GE Life Sciences, Pittsburgh, PA) conditioned with DCM and brought to a final volume of 5-7 mL in DCM.

Lipid removal was accomplished using an ABC Laboratories automated gel permeation chromatography (GPC) system. The column (J2 Scientific, Columbia, MO) was packed with 70 g of SX3 biobeads swelled with DCM with a flow rate of 5 mL DCM\min was used for lipid removal. An initial 5 mL of DCM was injected followed by the sample. Each sample was run on a 65 minute cycle with a 25 minute dump time and 40 minute collection time. The collected fraction was reduced in volume to 2-3 mL using a Kuderna-Danish system. The remaining fraction was evaporated under nitrogen to dryness. Samples were reconstituted in ~225 µL of Acetonitrile and transferred to a glass insert which was placed in a -20˚C freezer for 30 minutes to further reduce any remaining lipid. After freezing, the samples were transferred to a pre-weighed analytical vial and evaporated under nitrogen to dryness. The vials were weighed to determine residue weights and the sample was reconstituted in Ethyl Acetate to a final volume of 100 µL. The lipid cleanup steps successfully reduced the final residue weight to a typical value of about 3 mg.

2.2.3 GC\MS Analysis and Quantification

Samples were analyzed on an Agilent 6890N\5937 GC\MS (Agilent, Santa Clara, CA) operating in selected ion monitoring (SIM) mode. The method parameters consisted of an initial oven temperature of 45°C with a 2 min hold ramp of 50°C/min to 225°C and 10°C/min to 300°C for 25 minutes. Sample quantification was carried out using a 6 point calibration curve ranging from 250 ppb to 8 ppb with an analyte mix containing the five congeners of interest (Accustandard, New Haven, CT, BDE-47, -99, -100, -153 and -154). The method detection limits were determined using a surrogate matrix (corn oil) spiked at 25 ng/g. Seven replicates were run and the MDL was estimated as the standard deviation of the recovery values for the seven

replicates times 1.943 (the single sided "t" value at $p \leq 0.05$ with 6 degrees of freedom). The individual congener detection limits in ng/g were: 2.66 BDE-47, 2.76 BDE-99, 3.40 BDE-100, 3.80 BDE-153, and 4.19 BDE-154.

Quality assurance procedures consisted of a series of solvent blanks (n=5), method blanks run every five samples (n=4), and matrix spikes (n=4, blubber with known background concentrations). There was no evidence of target compounds in any solvent or method blanks. The mean recovery, based on the matrix spikes, for BDE-47, 99, 100, 153, and 154 was 101.66 ± 24.28%, 111.08 ± 7.29%, 55.09 ± 7.72%, 128.89 ± 8.21%, and 108.02 ± 4.39%, respectively. A sample of pilot whale blubber (SRM 1945) was obtained from the National Institute of Standards and Technology (NIST) and used as the standard reference material. The coefficients of variation (n = 4) for all detected PBDEs ranged from 4% to 9% with detected concentrations of all congeners being within 25% of the expected values.

2.2.4 Data Analyses

Concentrations for each congener were determined by multiplying the extract concentration from instrument quantification by a dilution factor and dividing by the initial mass. Any concentrations that fell below the MDL were excluded from further analysis. Individual congener values were added together to determine a ΣPBDE for each individual. A percent of the total, the proportion each congener represented of the ΣPBDE was also calculated for each of the five congeners per individual. Means by species were determined for each individual congener, congener group (tetra-, penta- and hexa-), congener percentages and ΣPBDEs.

Normality of the data was tested using the Shapiro-Wilk test and an outlier test was run

using JMP 12 (SAS Institute Inc., Cary, NC). All outliers (n=1) were excluded from analyses. ΣPBDEs, individual congener concentrations, and individual congener percent of total were compared between species using a Wilcoxon test (α = 0.05). ΣPBDEs and individual congener concentrations were also compared for sex and stranding condition code using a Wilcoxon test (α = 0.05).

2.3 Results and Discussion

2.3.1 PBDE Concentrations in Blubber

The goal of this study was to determine accumulation levels and congener profiles of PBDEs in stranded harp and hooded seals. Of the five congeners targeted in this study, three (BDE-47, 99, and 100) were found in all 30 samples at detectable levels. BDE-153 was found in 27 samples at detectable levels, while BDE-154 was found at detectable levels in 8 samples. The mean ΣPBDE concentration in harp seals was 70.55 ± 33.59 ng/g ww (range: 24.66-205.72, n=21) and in hooded seals was 94.28 ± 42.65 ng/g ww (range: 39.29-149.74, n=9). The highest ΣPBDE concentration was found in a female yearling harp seal (205.72 ng\g ww). No significant difference was observed for ΣPBDE between species (X^2= 1.87, df= 1, p= 0.17, Figure 2.1). The concentrations of PBDEs seen in stranded harp and hooded seals in this study are similar to those reported in other phocid species (Frouin et al., 2011; Ikonomou and Addison, 2008; Law et al., 2003; Shaw et al., 2008; Wolkers et al., 2006). Harp seals in this study had overall higher PBDE levels than those reported in a previous study and sampled from the same population (21 ± 3.4 ng/g; Frouin et al., 2011), but that study looked only at pups of different ages whereas the samples in this study were from yearlings and adults (Frouin et al., 2011). Concentrations of

PBDEs in hooded seals are consistent with previous studies that found ΣPBDE levels ranging from 31.9-148 ng/g lw (Rotander et al., 2012a; Wolkers et al., 2006).

These species have very similar life history strategies. Both species have similar reproductive strategies relying on seasonal ice cover and wean their pups in short time frames with the pups being a similar size at weaning (harp seals: 34 kg and hooded seals: 40 kg) (Lavigne and Kovacs, 1998). Adults of these species vary in size with hooded seals, both male and female (300 kg and 160 kg, respectively), being larger than harp seals (male and female: 130 kg) (Lavigne and Kovacs, 1998). Most of the individuals in this study though were young individuals which are more similar in size between the species than adults. Similarity in growth rate and size at weaning of young animals (Lavigne and Kovacs, 1998) could also explain why no difference was seen in the ΣPBDE levels between the species in this study.

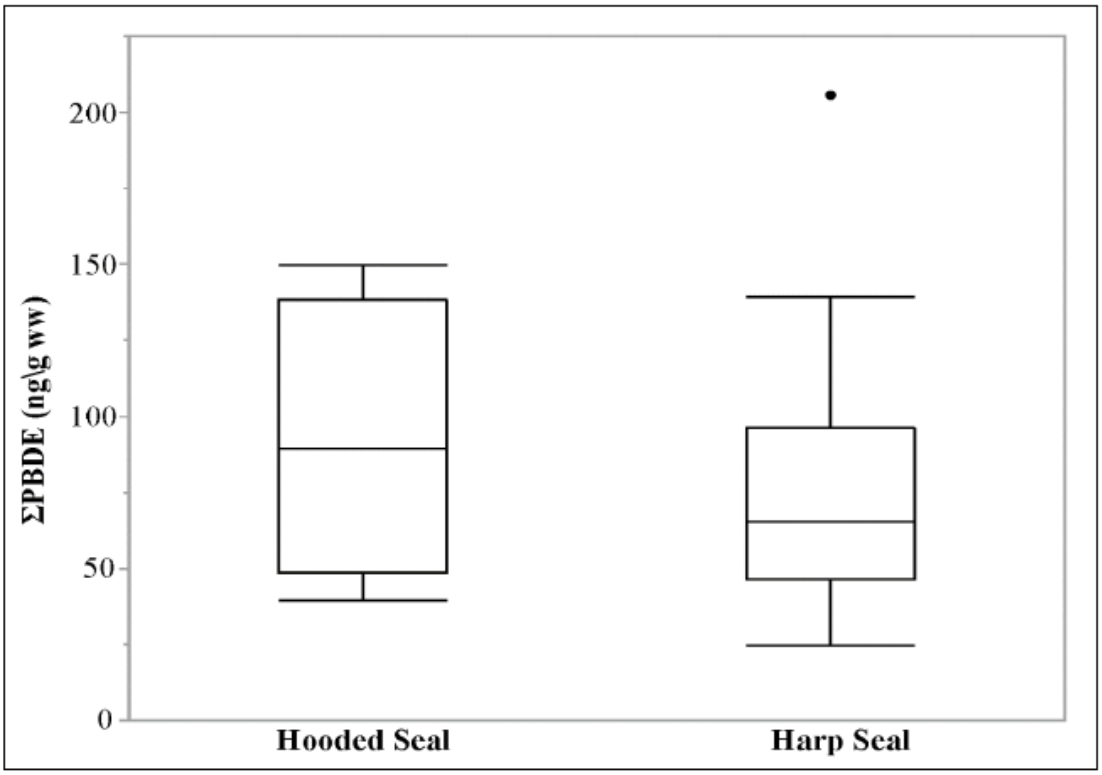

Figure 2.1: Median ΣPBDEs (25-75 % quantiles) for harp (n=21) and hooded (n=9) seals in ng/g ww. • represents outliers

A mean concentration and a mean percent of the total was calculated for each congener to determine a congener profile for each species. The concentration of only one congener, BDE-154, was significantly higher in harp seals compared to hooded seals (X^2= 4.2, df= 1, p= 0.04, Table 2.2).

Table 2.2: Individual congener means (±SD) in ng/g ww by species and stranding condition code.
*-denotes significance between species

	ΣPBDEs	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154
Species						
Harp Seals	70.55 ± 33.59	36.43 ± 16.03	12.03 ± 7.13	11.66 ± 6.61	9.32 ± 5.52	8.11 ± 2.24
Hooded Seals	94.28 ± 42.65	36.90 ± 18.04	22.26 ± 16.54	22.48 ± 17.95	11.71 ± 3.36	4.21 ± 0.04
Condition Code						
Code 1	100.76 ± 46.05	40.96 ± 14.56	17.44 ± 7.79*	17.96 ± 10.01*	11.29 ± 5.14	7.69 ± 2.59
Code 3	65.75 ± 40.13	34.15 ± 19.82	10.62 ± 6.45	8.29 ± 7.77	7.97 ± 3.57	6.15 ± 2.29

However, this congener was only found at a detectable level in a small sample size (n=2) for hooded seals. There was no significant difference in concentration between species for any other congeners. However, the mean percent of the total that each congener represented varied between species. For both species, BDE-47 represented the largest portion of the ΣPBDEs (harp: 54.22%, hooded: 39.85%, Figure 2.2). When compared between species, the percent of total for BDE-47 was significantly higher in harp seals (X^2= 6.72, df= 1, p= 0.0095, Figure 2.2), while BDE-99 was significantly higher in hooded seals (X^2= 4.11, df= 1, p= 0.0043, Figure 2.2). Congeners were grouped by level of bromination into three categories: tetra-, penta-, and hexa-. Only one congener group mean, penta- (BDE-99 and -100), was significantly different between the species being higher in hooded seals (X^2= 5.55, df = 1, p= 0.019, Table

2.S1). Overall, lower brominated congeners\groups account for a majority of the ΣPBDEs in both species.

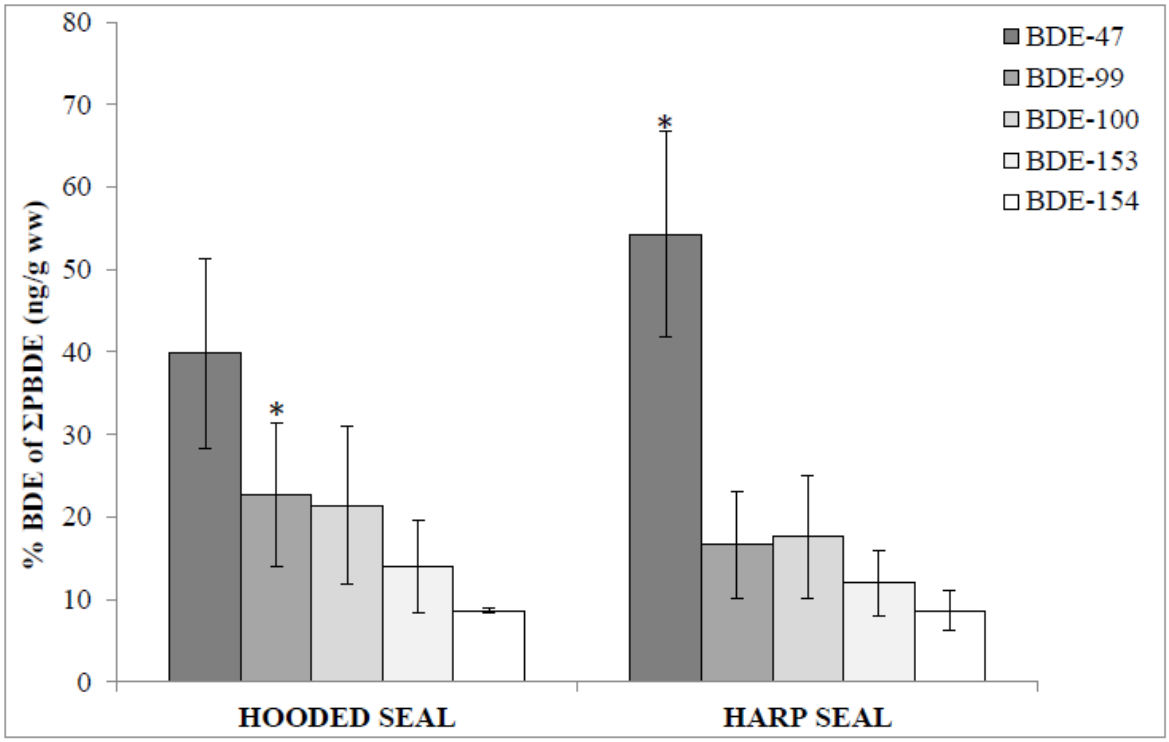

Figure 2.2: Congener profiles, shown as percent of ΣPBDEs (±1SD), for each species.
*-denotes significant difference between species for that congener ($p < 0.01$).

The congener profiles observed in this study are consistent with other studies on both harp and hooded seals with BDE-47 being the dominant congener (Frouin et al., 2011; Wolkers et al., 2006). The results are also similar with previous studies in that tissue accumulation levels decrease with increasing bromination (Frouin et al., 2011). Similar to patterns seen in this study, a previous study looking at both ringed seals, a species similar in size and diet to harp seals, and hooded seals, showed that in some years BDE-99 represented a higher percent of the total than BDE-47 in hooded seals and was always higher than in ringed seals (Rotander et al., 2012a). Hooded seals tend to stay further offshore, frequent land more rarely, and feed in deeper waters than harp seals, but most of their ranges and some pupping patches overlap

(Lavigne and Kovacs, 1998). Some of the differences in percent of total could be contributed to the dietary differences. Hooded seals feed on a more demersal diet, mainly Greenland halibut and redfish, whereas harp seals prey mainly on capelin and cod (Hammill and Stenson, 2000). Both of the hooded seal prey species are long-lived, slow growing species that feed on other fish and crustaceans. Harp seal diets in this region are composed mostly of capelin which feed on plankton and krill (Hammill and Stenson, 2000).

Both of these species complete long migration cycles which allows for exposure from multiple sources which could explain some differences in PBDE concentrations reported between studies and sampling locations (Lavigne and Kovacs, 1998; Rotander et al., 2012a). Hooded seals have a different migration pattern than harp seals and spend more time on both coasts of Greenland. This difference between the species could also explain some of the differences in congener profiles due to exposure from different sources of PBDEs (Rotander et al., 2012a; Wolkers et al., 2006). Both species reside in the St. Lawrence gulf and estuary during non-migratory periods (Frouin et al., 2011). These are areas with higher contaminant levels due to the drainage of the Great Lakes basin with a gradient of decreasing concentrations from the estuary out into the gulf (Hobbs et al., 2002). Differences in residence times of both of these areas between harp and hooded seals could potentially impact the congeners of PBDEs that they are exposed to. The combination of dietary differences, migration patterns, and residency times could be why though similar in ΣPBDE levels that harp and hooded seals have differences in congener profiles.

2.3.2 Effects of Demographics on PBDE Concentrations

The only significant difference in PBDE concentrations seen between species was in the

concentration of one congener (BDE-154) with low samples size; thus species were grouped together for all demographic analyses. All but three of the seals sampled were yearlings (Table 2.1). Yearlings, or young-of-the-year seals, are those from the previous pupping season that have been weaned and begun their first migration (Lavigne and Kovacs, 1998; Riedman, 1990). All three adult samples fell within the ΣPBDE distribution of the yearlings so there was no significant difference in PBDE concentration between age classes. The mean lengths of yearlings and adults were 102.78 ± 7.25 cm and 155 ± 14 cm, respectively. There were no significant relationships between length and any PBDE concentrations (ΣPBDE or individual congeners). When species were combined, the samples were split evenly between male and female. Mean ΣPBDEs concentrations for females and males were 78.23 ± 37.40 and 82.10 ± 42.88 ng/g ww, respectively. There were no significant differences between sexes in concentrations of any of the congeners or ΣPBDEs (Table 2.S1).

In yearlings, a large part of the contaminant load is representative of what was maternally transferred and varies based on the diet and residency time in the St. Lawrence gulf and estuary of the mother (Ikonomou and Addison, 2008; Shaw et al., 2008). Females can transfer up to 44% of their ΣPBDEs to resulting in similar congener profiles between mother and pup (Ikonomou and Addison, 2008). Yearlings can also be exposed through diet as they begin to migrate and forage on more typical prey species (Shaw et al., 2008), which could potentially explain why adults and yearlings had similar PBDE levels. Previous studies on young harbor seals have shown no differences between sexes (Shaw et al., 2008; Wolkers et al., 2006). Differences have been observed however between sexes in adult seals (both harbor and hooded seals), and that difference can change based on time of year (i.e. lactational transfer in

females) (Shaw et al., 2008; Wolkers et al., 2006).

The final demographic metric analyzed was stranding condition code (i.e. the condition in which the seal was found, Table 2.S2). Due to the small sample size (Table 2.1) for most condition codes, only codes 1 (alive) and 3 (moderate decomposition) were used for analysis. Means for ΣPBDEs and individual congeners are shown in Table 2.2 for each code. Concentrations, both total and congener group, were higher in samples classified as code 1. ΣPBDEs compared between the two condition codes approached significance (X^2= 3.55, df =1, p = 0.0597). The two penta-BDEs (99 and 100) were significantly different between the two condition codes (99: X^2 = 3.99, df =1, p = 0.0456, 100: X^2 = 9.27, df =1, p = 0.0023). As mentioned previously, yearling seals are more likely to strand than adult seals (Soulen et al., 2013). The condition in which a stranded seal is found could potentially impact the levels of contaminants found. These results suggest that degradation of tissue affects levels of PBDEs. Lower concentrations of BDE-99 and -100 in the moderately decomposed individuals could also indicate that debromination of congeners was occurring as the tissue was breaking down (de Wit, 2002; Rahman et al., 2001). Seals that have stranded may have mobilized their blubber stores for energy before stranding. This mobilization of blubber could decrease the total blubber layer in both code 1 and 3 animals. Code 3 animals may have mobilized more of their blubber stores than code 1, which could translate to lower concentrations of lipophilic compounds being measured in moderately decomposed individuals.

2.4 Conclusions

Harp and hooded seal pups and yearlings initially rely on a smaller layer of blubber and fur for energy and temperature regulation (Lavigne and Kovacs, 1998). In a quickly changing

system, like the ice packs of the Arctic, young seals may be forced to fend for themselves earlier than expected (Johnston et al., 2012). This could lead to the mobilization of blubber stores and changing body condition. Poor body condition, like that seen in stranded seals, can impact the circulating levels of contaminants like PBDEs that have been remobilized when blubber stores are used (Lehnert et al., 2016).

Stranded animals are usually considered immune compromised which could be related to contaminant-induced impacts on the animals' immune system (Jepson et al., 1999; Jepson et al., 2005; Kuiken et al., 1994). For yearling seals that are still developing a full immune system, susceptibility to disease can be high and efficient use of energy is important (Frouin et al., 2010; Frouin et al., 2011; Hall et al., 2003). Stranded grey seal pups have shown elevated granulocyte levels in conjunction with elevated xenobiotic biomarkers which can indicate contaminant exposure through lactational transfer, stress, and dehydration (Lehnert et al., 2014). A previous study on harbor seal immune cells also indicated that PBDEs could cause oxidative damage to granulocytes which could affect the ability to fight disease (Frouin et al., 2010). Potential links between PBDE exposure and thyroid hormones in yearling grey seals could also indicate impacts on the development of the immune system in young seals (Hall et al., 2003). If the immune system of yearlings is compromised from the beginning, then the chances of stranding may increase.

Yearlings are the most dominant age class to strand in all years, but are especially vulnerable in years of lighter ice cover (Soulen et al., 2013). As mentioned above, yearlings being forced into the water sooner causing them to fend for themselves can increase physiological stress on the animal and change the use of energy. Decreases in ice cover can also

lead to young seals using sub-prime habitat (Soulen et al., 2013), where exposure to disease increases for seals with potential immune suppression from contaminants like PBDEs. The contaminant burden yearling seals may have coupled with physiological stress and immune suppression could together explain why seals may be stranding. Threshold effects for PBDEs in marine mammals are relatively unknown even though effects have been seen (Shaw et al., 2008). Levels of both thyroid hormones have found to be correlated with ΣPBDE concentrations in grey seals (Hall et al., 2003) and thymic atrophy was observed in conjunction with ΣPBDEs in stranded harbor porpoises (Beineke et al., 2005). Recent studies suggest that toxic effects of PBDEs both alone and in mixtures may occur at lower levels than previously thought (Shaw et al., 2007; Van den Berg et al., 2006). Arctic marine mammals are unique, compared to other non-Arctic species, in that they inhabit a quickly changing environment in which they are exposed to a mixture of environmental pollutants from the beginning along with other environmental stressors which can make understanding the impact of accumulating contaminants, like PBDEs, difficult.

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CHAPTER 3

PERSISTENT ORGANIC POLLUTANT EXPOSURE AND ASSOCIATIONS WITH GENE EXPRESSION IN NORTHERN FUR SEALS FROM ST. PAUL ISLAND, ALASKA

3.1 Introduction

Persistent organic pollutants (POPs) are ubiquitous environmental contaminants produced either directly or indirectly through human activity. They have long environmental half-lives, are highly lipophilic, and are often volatile allowing for long-term atmospheric transport (Hutchinson and Simmonds, 1994). This allows POPs to persist in the environment, especially in cold ecosystems, where they can biomagnify through the food chain (Simonich and Hites, 1995; Wania and Dugani, 2003). Increased concentrations of POPs in biota inhabiting polar regions can indicate a growing environmental problem (de Wit et al., 2006; de Wit and Muir, 2010). High levels of persistent contaminants, including polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCPs), are being observed in marine systems making it important to understand the impact on high trophic level species like marine mammals.

Marine mammals are long-lived species with limited capacity to detoxify persistent chemicals (Ross, 2000; Tanabe et al., 1983). Since the 1960s, studies have shown that marine mammals accumulate POPs at potentially toxic concentrations particularly in blubber (Jensen, 1966). In some species of marine mammals, especially sub-Arctic and Arctic seals, the blubber can contain upwards of 90% of the POP accumulation (Tanabe et al., 1981). Due to their dependence on blubber as an energy source at different life history stages, marine mammals are susceptible to high contaminant loads (Wolkers et al., 2006). Exposure to complex mixtures

of these compounds can lead to reproductive, immunological, and endocrine issues (de Swart, 1995; De Swart et al., 1996). Marine mammals are sentinel species for the systems that they inhabit (Cipro et al., 2012) making them good indicators of environmental contamination by POPs (Ross, 2000).

Northern fur seals (*Callorhinus ursinus*) are a subarctic species found across the northern Pacific Ocean from the Sea of Japan to the Channel Islands (Nowak and Walker, 2003; Riedman, 1990). They are the only species of fur seal in the Northern Hemisphere and more pelagic than most fur seals spending 9-10 months at sea (Nowak and Walker, 2003; Riedman, 1990). Northern fur seals breed on a number of small islands, including St. Paul Island and St. George Island in the Pribilof Islands in the eastern Bering Sea where the largest aggregations occur (Nowak and Walker, 2003). While on land, both male and female seals intermittently fast, relying on built up blubber stores for energy and milk production (Muto et al., 2020; Riedman, 1990). The populations in the Pribilof Islands are managed as a depleted stock and are of conservation concern, in part due to their importance as a food source for the local Aleut communities (Muto et al., 2020). Threats to northern fur seals range from changes in food availability and entanglement in fishing gear to environmental contaminants (Muto et al., 2020).

Exposure to increasing numbers and mixtures of contaminants has the potential to affect the immune, endocrine, and other systems which can leave northern fur seals more susceptible to disease and potentially reduce overall survival and fitness (Muto et al., 2020). POP concentrations have been measured in northern fur seals across their range. Kajiwara et al. (Kajiwara et al., 2004) found some of the highest concentrations of DDTs, PCBs, PBDEs, and

other OCPs in northern fur seals sampled in Japan, but showed a decrease in concentrations from the 1970s. Within the Pribilof Islands, DDT and PCB concentrations of over 1 ppm have been observed while concentrations of PBDEs have been seen at lower concentrations than other contaminants (< 25 ppb) (Lee et al., 1996; Reiner et al., 2016). Studies have shown that exposure to POPs, especially PCBs, has a potential impact on immune and endocrine function in northern fur seal pups (Beckmen et al., 2003; Beckmen et al., 1999; Mori et al., 2008; Mori et al., 2006).

Within the marine ecosystem, a wide variety of biomarkers are used to assess these exposure-effect relationships. Metallothioneins have long been used as biomarkers of heavy metal exposure in marine invertebrates and fish (Amiard et al., 2006; Viarengo et al., 1999; Walker et al., 2014). The inhibition of acetylcholinesterase activity after exposure to organophosphate and carbamate pesticides has also been a widely used biomarker in marine systems (Lionetto et al., 2013; Rickwood and Galloway, 2004). Impacts on the activity of ethoxyresorufin-O-deethylase (EROD), a function of the cytochrome P450 1A subfamily, is used as a biomarker of exposure to PAH, dioxin, and other contaminants across the marine food web. Molecular biomarkers can be used to help better understand the relationship between exposure and potential impacts.

The aryl hydrocarbon receptor (AhR) and its dimerization partner the aryl hydrocarbon receptor nuclear translocator (ARNT), can be activated by environmental contaminants and are a pathway for the metabolism of contaminants (Beischlag et al., 2008; Lehnert et al., 2014; Stange and Veldhoen, 2013). Biomarkers that are components of contaminant metabolism pathways, like AhR and ARNT, are useful in that they can be sensitive early warning indicators

of exposure to environmental contaminants that can have impacts at the cellular level (Gil and Pla, 2001). Cytochrome P450s (CYP) are monooxygenase enzymes that are important in the metabolism of both endogenous and exogenous compounds (Chiba et al., 2002; O'Hara and O'Shea, 2001). In marine mammals, the potential for interactions between different contaminants and enzymes is very complex due to the fact that various contaminants can act as enzyme inducers, inhibitors, or substrates, and the intermediate metabolites formed can be even more toxic (O'Hara and O'Shea, 2001). The CYP1A subfamily of enzymes is often mediated by AhR and in known to be induced by a number of contaminants (Addison and Brodie, 1984; O'Hara and O'Shea, 2001; O'Shea, 1999). Continuous induction or inhibition of CYP enzymes can potentially impact the metabolism, accumulation, and mechanisms of both contaminants as well as endogenous compounds which can in turn alter the physiology of exposed organisms (Chiba et al., 2002). Previous studies in marine mammals have shown that CYP1A and other CYP enzymes are correlated with contaminant concentrations, including PCBs (Troisi and Mason, 2000; Wolkers et al., 1998; Wolkers et al., 1999).

Thyroid hormones (TH) are important in the regulation of numerous cellular functions including metabolism through the regulation of gene transcription rates (Martinez et al., 2013; Reitman et al., 1999). Studies done on laboratory organisms have suggested that compounds, like PCBs, can affect thyroid receptor activity (Zoeller, 2005). THs interact with cellular receptors as signaling molecules, including thyroid receptor alpha (TR-α), and alter their activation and repression activities (Chiba et al., 2001; Hall et al., 1998; Tabuchi et al., 2006). Triiodothyronine (T_3) is the more bioactive form involved in cellular regulation and is dependent on the deiodination of thyroxine (T_4) at the target tissue through deiodinases (DI 1) (Martinez et

al., 2013). During periods of fasting, DI 1 is increased to aid in the suppression of cellular metabolism to decrease energetic burdens that can be imposed during reduced energy intake through the production of reverse T_3 (LoPresti et al., 1991; Martinez et al., 2013).

The goals of this study were to determine the accumulation of POPs in the blubber of northern fur seals and to correlate concentrations of total POPs as well as individual compounds with mRNA expression of relevant biomarkers: AhR, ARNT, CYP1A, TR-α, and DI1. These findings are important because northern fur seals are reliant on blubber as an energy source during times of reduced food intake. Changes in the regulation of blubber metabolism pathways from contaminant exposure can potentially have a significant impact. Increasing concentrations of contaminants can also potentially affect growth, development, and immune health which could have impacts on the overall survival of the population.

3.2 Methods

3.2.1 Sample Collection

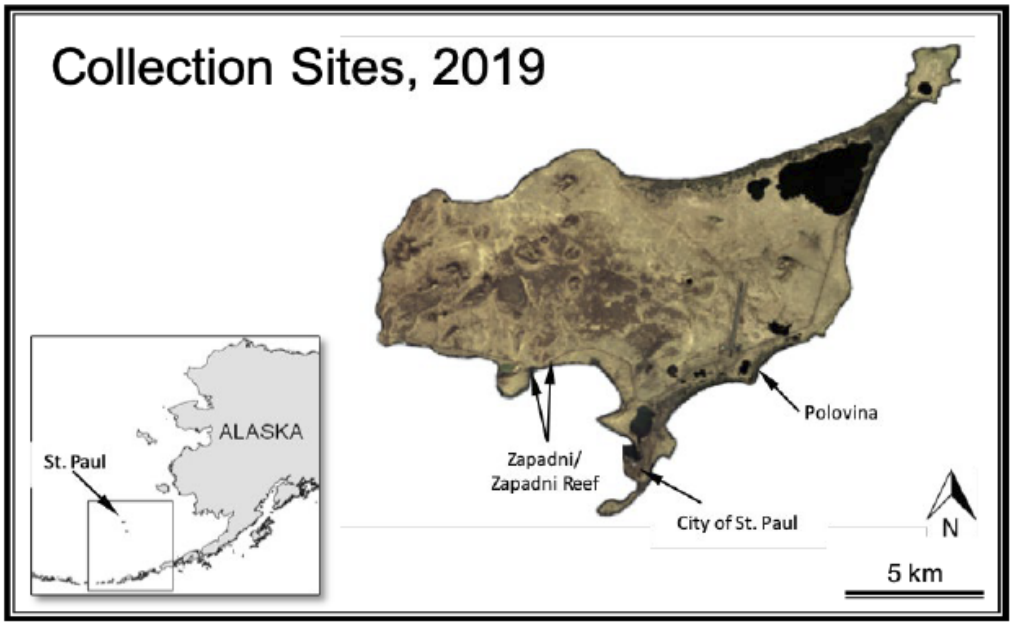

Figure 3.1: Map of the sample locations around St. Paul Island, Alaska, USA.

Northern fur seals were sampled on St. Paul Island, Alaska during the yearly harvest from June 23 – August 8, 2019. Blubber tissue was collected from 16 sub-adult male northern fur seals. Sampling location as well as demographic data (sex, age, body length, and blubber depth) were collected for each individual (Figure 3.1). Tissue samples were stored at -80°C before being subsampled for analysis. All animal handling and sampling protocols were conducted under National Marine Fisheries Permit No. 19436 and all samples were processed under University of North Texas IACUC Tissue Use Permit 14009.

3.2.2 Sample Extraction

Blubber tissue was sub-sampled (350±10 mg), weighed in a clean weight boat, and placed into a mortar with ~4-5 g of sodium sulfate. The homogenate was then transferred to a 50 mL Falcon tube and 25 mL of a 50:50 by volume mix of dichloromethane (DCM):hexane (Acros Organics, Pittsburgh, PA) was added. The sample was vortexed on high for 1 minute followed by centrifugation at 3,500 rpm for 5 minutes. The organic layer was transferred into a clean, pre-weighed 50 mL Falcon tube and the process was repeated with a second 25 mL for a final volume of ~50 mL. The extract was evaporated under nitrogen in a water bath (~40°C) until all solvent was removed to obtain an estimated lipid mass for each sample. The final mass of the tube was recorded and 5 mL of DCM was added. Two fluorinated-PBDEs and one methoxylated-PBDE (FBDE-47, FBDE-154, and 3MeO-28, Accustandard, New Haven, CT), two carbon labelled PCBs (13C PCB-28 and 13C PCB-209, Cambridge Isotope Laboratories, Inc, Tewksbury, MA), and a mix of carbon labelled DDTs (C13-DDD, C13-DDE, and C13-DDT, Cambridge Isotope Laboratories, Inc, Tewksbury, MA) were added as internal standards (100 ng/g each) to the extract.

An automated gel permeation chromatography (GPC, ABC Laboratories) system was used for lipid removal. The column (J2 Scientific, Columbia, MO) was packed with 70 g of SX3 biobeads swelled with DCM at a flow rate of 5 mL/min. For each extract, an initial 5 mL of DCM was injected followed by the 5 mL of sample, and run on a 65 minute cycle (25 minute dump time and 40 minute collect time). The collected sample was reduced to 2-3 mL using a Kuderna-Danish system and evaporated to dryness under nitrogen. Samples were reconstituted in 225 µL of Acetonitrile, transferred to a glass insert, and placed at -20°C. After 30 minutes, the sample was transferred to a pre-weighed analytical vial and evaporated to dryness under nitrogen. Vials were weighed to determine residue weights and the sample was reconstituted in 100 µL of Ethyl Acetate.

3.2.3 GC/MS Analysis and Quantification

Blubber extracts were analyzed on a Thermo Scientific Trace 1310/TSQ8000Evo GC/MS (Thermo Fisher Scientific, Waltham, MA) using selective reaction monitoring mode utilizing target and transition ions for quantification and identification (Table 3.S1). All samples were analyzed for five PBDEs, two methoxylated PBDEs (MeO-BDE), fifteen PCBs, and ten OCPs (see a full list in Table 3.S1). Sample quantification was carried out using the following calibration curves: eight point curve ranging from 500 ppb to 4 ppb for PBDEs, nine point curve ranging from 500 ppb to 2 ppb for PCBs, and a ten point curve ranging from 1000 ppb to 2 ppb for OCPs. Method detection limits (MDLs) were determined using a surrogate matrix (corn oil) spiked at 30 ng/g. The MDL was estimated as the standard deviation of the recovery values for seven replicates times 1.94. Individual compound/congener detection limits can be found in Table 3.S1.

Quality assurance consisted of solvent blanks run after every two samples and method blanks were processed every four samples (n=4). There was no evidence of any target compounds in either the solvent blanks or method blanks. A sample of pilot whale blubber (SRM 1945) obtained from the National Institute of Standards and Technology (NIST) was used as a standard reference material for all classes of compounds. The coefficients of variation (n=4) for all detected PBDEs ranged from 5.6 – 21.4%, for all detected PCBs ranged from 4.3 – 13.5%, and for all detected OCPs ranged from 6.4 – 25%.

3.2.4 RNA Isolation and qPCR

Frozen blubber tissue (75-100 mg) was extracted for total RNA using TRI reagent (Sigma Aldrich), following the manufacturer's instructions. Following extraction, RNA samples were treated with DNAse I (Qiagen) before being run through RNeasy MinElute Cleanup columns (Qiagen). Following isolation, RNA was analyzed on a NanoDrop (Thermofisher). RNA (1 ug) was reverse transcribed to cDNA using iScript Reverse Transcription Supermix for RT-qPCR (Bio-Rad), following manufacturer's instructions. After completion, cDNA was stored at -20°C until qPCR was conducted. qPCR was performed using SsoAdvanced Universal SYBR Green Supermix (Bio-Rad) on an AriaMX Real-time PCR System (Agilent). Each reaction contained 100 ng of cDNA with specific primer pairs (10µM) for the gene of interest and all reactions were run in triplicate. The following cycling conditions were used: denaturation for 2 minutes at 95°C, with 40 cycles of a 10 second denaturation at 95°C and an annealing and extension for 60 seconds at the optimal annealing temperature for each primer. A melt curve was carried out from 55 to 95°C in increments of 0.5°C. Primer efficiencies were calculated and all genes were normalized to the housekeeping gene GADPH (ΔCT). Changes in gene expression were assessed using the

$2^{-\Delta\Delta CT}$ method using the individual with the highest ΔCT for each target gene as the control because it represented the individual with the lowest expression for that gene (Livak and Schmittgen, 2001).

3.2.5 Data Analyses

For all compounds, concentrations were determined by multiplying the concentration from instrument quantification by the dilution factor and dividing by the sample lipid mass. Any concentrations found to fall below the MDL were excluded from further analyses. Contaminant concentrations and gene expression levels were tested for normality and natural log (ln) transformed for all analyses. An ANOVA with a Tukey-Kramer post-hoc was used to compare ΣPBDE, ΣPCB, and ΣOCP concentrations. Individual compound concentrations, sum concentrations, and demographic factors were compared using linear regressions. A least squares full factorial linear regression model was performed to assess the interaction between sum concentrations (PBDE, PCB, OCP, and POP) and demographic factors. Gene expression levels and contaminant concentrations, both individual and sum, were also compared using linear regression. A principal component analysis including all contaminant, demographic, and gene expression data was performed to observe the relationship between all factors. An alpha of 0.05 was used to determine significance. All statistical analyses were done using JMP 14 (SAS Institute, 1989-2007).

3.3 Results

3.3.1 Contaminant Concentrations in Blubber

The ΣPBDE mean (±1SD) concentration was 23.04 ± 15.18 ng/g lw (range: 12.63-75.48)

with BDE-47 representing the highest concentrations. Mean ΣMeO-BDE was 16.81 ± 7.46 ng/g lw (range: 9.02-31.88) with 6-MeO-47 representing over 60% of the ΣMeO-BDE for all samples. The mean total PBDE (ΣPBDE and ΣMeO-BDE) was 39.85 ± 19.59 ng/g lw (range: 21.13-101.64). For the PCB analyses, the mean ΣPCB concentration was 766.6 ± 459.09 ng/g lw (range: 159.5-1643.58; 13 congeners). PCBs 153 and 138 represented the highest concentrations and composed on average 70.9% of the ΣPCB. The mean ΣOCP concentration was 1073.08 ± 608.28 ng/g lw (range: 522.01-2594.61). The DDT metabolites, DDE and DDD, represented the majority of ΣOCP (on average 80%) with beta BHC composing 16.19%, on average. Concentrations of PBDEs were significantly lower than PCBs and OCPs ($F= 156.16$, $df= 2$, $p < 0.001$). ΣPOPs in northern fur seals had a mean concentration of 1879.5 ± 941.7 ng/g lw (range: 703.3-4339.8).

3.3.2 Effects of Demographics on Contaminant Concentrations

Mean length and blubber depth for the northern fur seal samples were 106.9 ± 4.34 cm (range: 99-119) and 23 ± 5.66 mm (range: 13-32), respectively. The mean percent lipid in northern fur seal blubber was 81.19 ± 7.72% (range: 68.61-94%). Only one compound (PCB 18) had a significant relationship ($r^2= 0.38$, $p= 0.011$) with seal body length. There were no significant relationships between contaminant concentrations and blubber depth ($p= 0.5$), location ($p= 0.21$), or sampling date ($p= 0.32$). Percent lipid was negatively correlated with concentrations of several individual compounds including: PCBs 18 ($r^2= 0.5$, $p= 0.002$), 153 ($r^2= 0.3$, $p= 0.03$), 180 ($r^2= 0.47$, $p=0.004$), 170 ($r^2= 0.36$, $p= 0.014$), and ΣPCB ($r^2= 0.31$, $p=0.024$); PBDEs 47 ($r^2= 0.3$, $p= 0.03$), 99 ($r^2= 0.52$, $p= 0.002$), 153 ($r^2= 0.47$, $p= 0.021$), ΣPBDE ($r^2= 0.44$, $p= 0.005$), 2MeO68 ($r^2= 0.26$, $p= 0.04$), 6MeO47 ($r^2= 0.4$, $p= 0.009$), ΣMeO-BDE ($r^2= 0.43$, $p= 0.006$), and total PBDE ($r^2= 0.58$, $p= 0.0006$); α-BHC ($r^2= 0.6$, $p=0.0004$), β-BHC ($r^2= 0.66$, $p= 0.0001$),

heptachlor epoxide (r^2= 0.4, *p*= 0.009), DDT (r^2= 0.38, *p*= 0.011), ΣBHC (r^2= 0.69, *p* < 0.001), and ΣPOPs (r^2= 0.4, *p*= 0.009)(Figure 3.2). A least squares full factorial linear regression model significantly explained the relationship between ΣPOP, body length, blubber depth, and percent lipid (r^2= 0.82, F= 5.21, *p*= 0.016). Percent lipid had the largest effects on ΣPOPs (F= 17.25, *p*=0.0032) followed by body length (F= 15.43, *p*= 0.0044). Blubber depth alone did not have a significant effect on ΣPOP concentrations (*p*= 0.63). For total PBDEs, ΣPCB, and ΣOCP, body length and percent lipid also had the largest effect on concentrations.

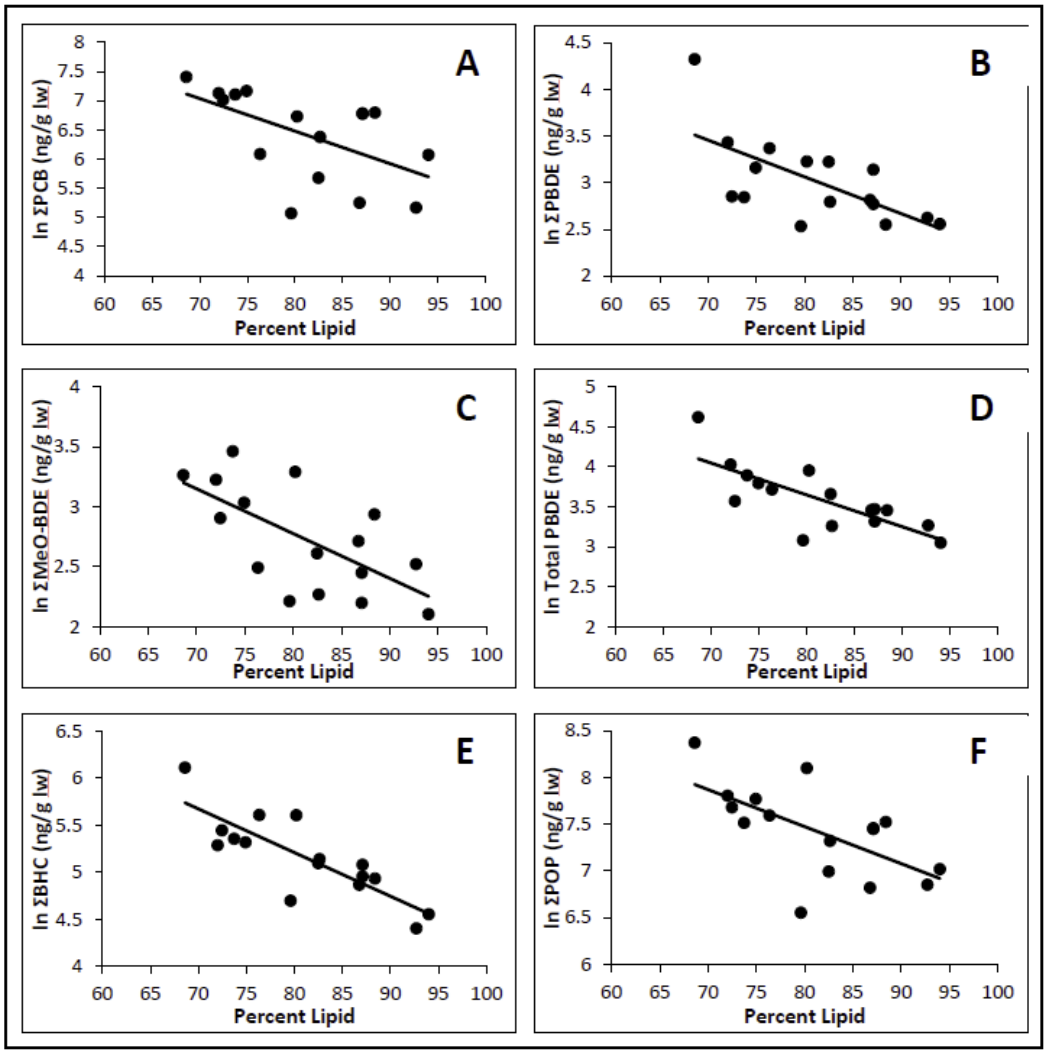

Figure 3.2: **The relationship between percent lipid and ln transformed contaminant concentrations for: A. ΣPCB (r^2= 0.31, *p*=0.024), B. ΣPBDE (r^2= 0.44, *p*= 0.005), C. ΣMeO-BDE (r^2= 0.43, *p*= 0.006), D. total PBDE (r^2= 0.58, *p*= 0.0006), E. ΣBHC (r^2= 0.69, *p* < 0.001), and F.ΣPOPs (r^2= 0.4, *p*= 0.009).**

3.3.3 Gene Expression and Contaminant Concentrations

AhR expression was positively correlated ($p < 0.05$) with concentrations of five PCB congeners (CB 52, 138, 153, 170, 180) as well as ΣPCBs, BDE 99, ΣPBDEs, α-BHC, and ΣPOPs (Table 3.1, Figure 3.3).

Table 3.1: Correlations between normalized gene expression for AhR and TR-α and contaminant concentrations, individual and sum.

	R^2	F-Ratio	*p*-value
AhR			
PCB 52	0.53	16.08	0.0013
PCB 138	0.3	5.95	0.029
PCB 153	0.27	5.16	0.04
PCB 170	0.29	5.68	0.03
PCB 180	0.28	5.58	0.03
ΣPCBs	0.35	7.38	0.017
PBDE 99	0.31	6.25	0.025
ΣPBDEs	0.25	4.71	0.048
α-BHC	0.25	4.6	0.05
ΣPOPs	0.31	6.15	0.026
TR-α			
PCB153	0.31	6.25	0.025
PCB 170	0.3	5.94	0.029
β-BHC	0.32	6.74	0.021
ΣBHC	0.31	6.3	0.025
Total PBDE	0.31	6.27	0.025
ΣPOPs	0.27	5.24	0.038

Concentrations of one PCB (CB 52) were positively correlated with ARNT expression (r^2= 0.32, F= 6.45, *p*= 0.02). Expression of CYP1A was negatively correlated with concentrations of PCB 105 (r^2= 0.45, F= 10.63, *p*= 0.0062) and PCB 180 (r^2= 0.26, F= 4.63, *p*= 0.05). DI 1 expression was negatively correlated with the concentrations of PCB 105 (r^2= 0.32, F= 6.44, *p*= 0.024). The

expression of TR-α was positively correlated ($p < 0.05$) with two PCB congeners (CB 153, 170), β-BHC, ΣBHC, total PBDEs, and ΣPOPs (Table 3.1). A significant, positive relationship was observed between the expression of AhR and ARNT (r^2=0.58, F= 19.06, p= 0.0006). There were no significant relationships between expression of CYP1A and expression of AhR or ARNT.

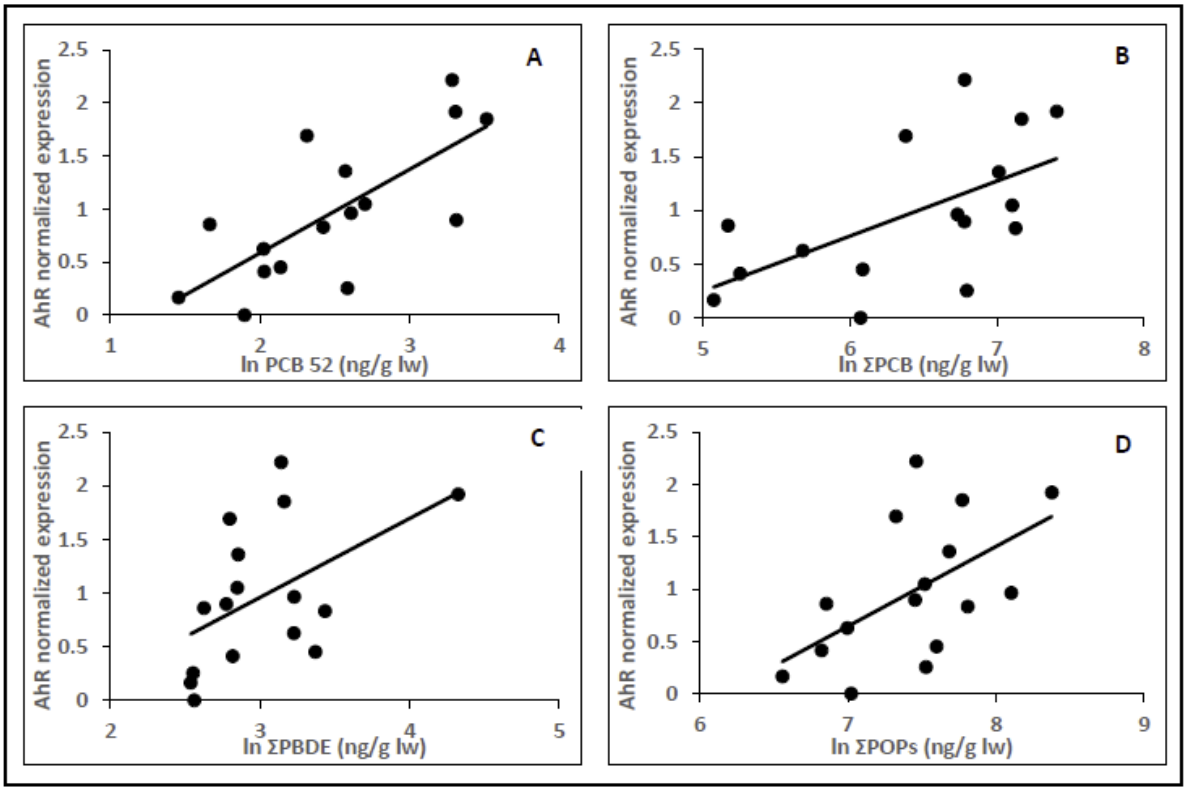

Figure 3.3: **The relationship between ln transformed contaminant concentrations and AhR normalized expression for: A. PCB 52 (r^2= 0.53, p= 0.0013), B. ΣPCB (r^2= 0.35, p= 0.017), C. ΣPBDE (r^2= 0.25, p= 0.048), and D. ΣPOP (r^2= 0.27, p= 0.038).**

Along PC1, there is a gradient representing a decrease in percent lipid and blubber depth, and an increase in contaminant load, AhR expression and ΣPCB concentrations. Additionally, there is a gradient along PC2 representing a decrease in ARNT expression and an increase in CYP1A, DI 1, body length and blubber depth. Principal components axes 1 and 2 account for 46% and 15.7% of the total variation in northern fur seal blubber samples, respectively (Figure 3.4).

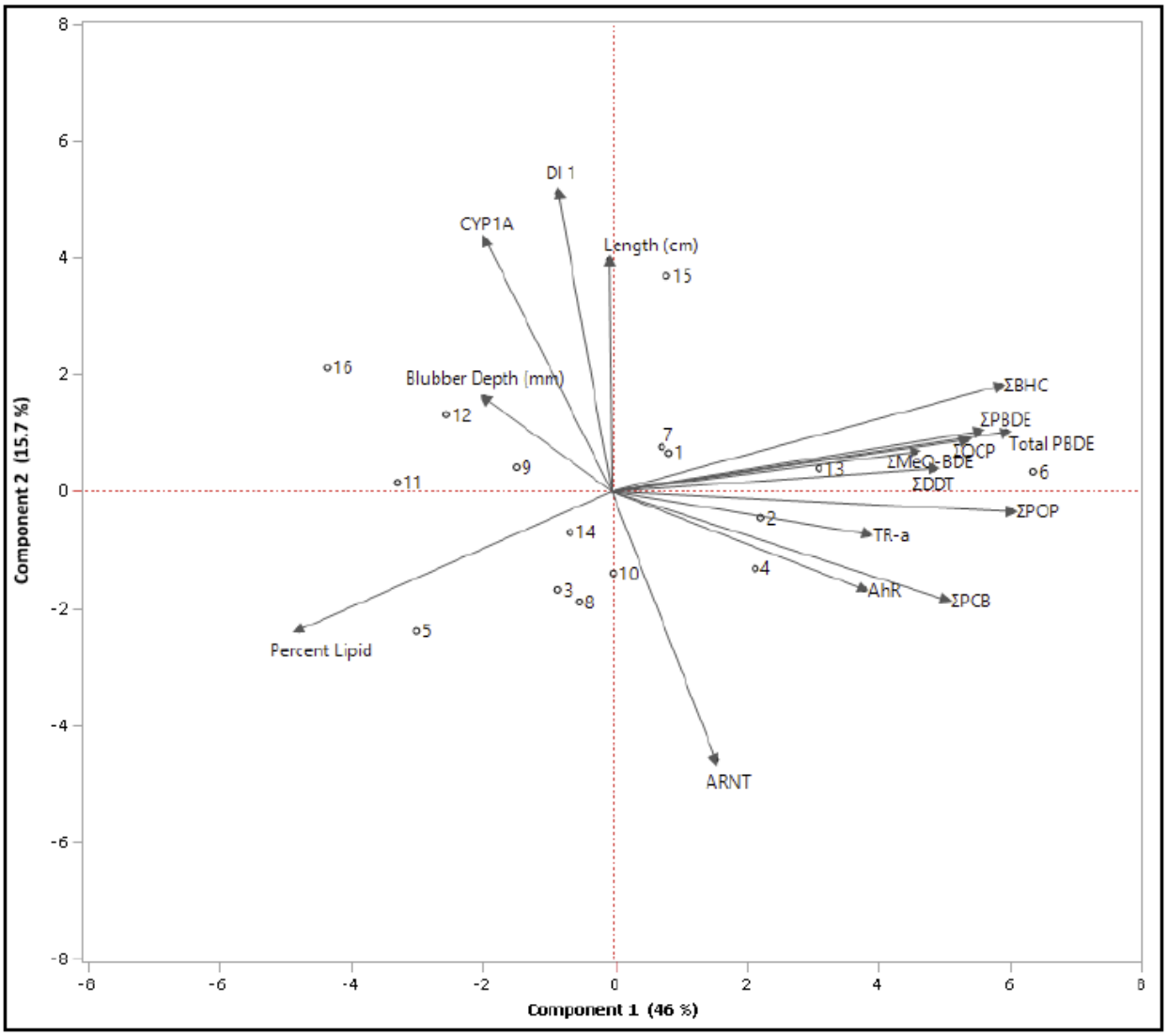

Figure 3.4: **PCA plot showing the relationships between contaminant groups, demographic factors, and normalized gene expression. Numbers represent individual northern fur seals.**

3.4 Discussion

The goals of this study were to determine accumulation of PBDEs, PCBs, and OCPs in blubber from northern fur seals and evaluate the relationship between contaminant concentrations and changes in gene expression of biomarkers to assess potential effects of exposure. Overall contaminant concentrations measured in this study are similar to those reported in other studies of northern fur seals. This is also the first study to measure the

accumulation of MeO-BDE in northern fur seals. Changes in gene expression for all biomarkers assessed were correlated with a number of individual compounds and sum concentrations. These results potentially indicate that northern fur seals are accumulating contaminants at concentrations that could pose a risk to their health.

ΣPBDE concentrations in northern fur seal blubber in this study are similar to those reported in previous studies on mature female seals from Japan (Kajiwara et al., 2004) and sub-adult males from St. Paul Island (Reiner et al., 2016). Northern fur seal females are able to offload a portion of their contaminant burdens yearly when they produce milk, which could potentially explain why mature females and sub-adults males have similar PBDE concentrations (Beckmen et al., 1999; Wolkers et al., 2006). Both this study and the Reiner et al. (2016) study used sub-adult male northern fur seals sampled from St. Paul Island, and even though the earlier study sampled over a broader temporal range (1987-2007) the mean ΣPBDE concentrations are similar (this study: 23.04 ± 15.18 ng/g lw; Reiner study: 26.3 ng/g lw) (Reiner et al., 2016). However, Reiner et al. (2016) did note that PBDE concentrations increased from 1987 to 2007 suggesting that more recently banned contaminants may be still be increasing in this area (Reiner et al., 2016).

ΣMeO-BDE concentrations are higher than those reported in previous studies of ringed seals (Kelly et al., 2008b; Rotander et al., 2012b) and hooded seals (Rotander et al., 2012b) sampled in the Arctic and California sea lions (Stapleton et al., 2006). Concentrations of 6-MeO-47 were either similar or higher than individual PBDE congeners, up 25 times higher for some PBDEs, which is similar to what has been reported in other marine mammals (Kelly et al., 2008b). Increasing concentrations of MeO-BDEs in Arctic and sub-Arctic pinniped species are

potentially due to either exposure to natural sources of MeO-BDE (ex. red algae, marine sponges, cyanobacteria) or increased biotransformation after uptake (Kelly et al., 2008b; Rotander et al., 2012b). There is little known about the toxicity of MeO-BDEs. Hu et al. (2011) found that both 2-MeO-68 and 6-MeO-47 were antiandrogens and possessed estrogenic activity with 6-MeO-BDE being more potent (Hu et al., 2011). In grey seals, MeO-BDEs were positively correlated with circulating levels of vitamin A indicating that they may have an effect on vitamin A homeostasis (Vanden Berghe et al., 2013).

The ΣPCBs determined in this study are similar to those reported in juvenile male northern fur seals from St. Paul Island sampled in the early 2000s (Wang et al., 2010), but lower than previous studies of sub-adult males from St. Paul Island sampled from the 1980s and 1990s (Loughlin et al., 2002; Reiner et al., 2016). Reiner et al. (2016) did not report any significant temporal trends for PCB concentrations, which could suggest that PCB concentrations in St. Paul Island may be remaining constant or declining slightly (Reiner et al., 2016). ΣDDTs (842.58 ± 500.95 ng/g lw) are lower than what has been previously reported in northern fur seals from St. Paul Island (Loughlin et al., 2002; Reiner et al., 2016; Wang et al., 2010). For other OCPs, ΣHCH concentrations are similar or higher than previous studies on northern fur seals sampled in the 2000s, but lower than those sampled in the 1980s and 1990s (Reiner et al., 2016; Wang et al., 2010). Together, these results suggest that concentrations of legacy POPs in sub-adult male northern fur seals from St. Paul Island are declining or remaining constant. This is consistent with Riget et al. (2010) who reported decreases in legacy POPs in Arctic biota, including ringed seals across their range (Riget et al., 2010).

The best predictor of contaminant concentrations in northern fur seal blubber was the

lipid content of the sample. The individual with the highest contaminant concentrations (total PBDEs, ΣPCBs, ΣOCPs, ΣPOPs) had the lowest percent lipid (68.6%) and the shallowest blubber depth (12 mm). Northern fur seals fast when migrating to breeding grounds and intermittently fast while at the colony (Muto et al., 2020; Riedman, 1990). During this time they rely on blubber stores as a source of energy. While it is thought that some contaminants are released into the blood stream when blubber is mobilized, others are likely to continue to be stored and therefore not likely to be bioavailable (Ikonomou and Addison, 2008; Wolkers et al., 2006). The negative relationship between contaminants and percent lipid in this study could be indicative of contaminants concentrating in the remaining blubber tissue during fasting periods rather than being mobilized (O'Hara and O'Shea, 2001).

The AhR pathway regulates the metabolism of contaminants, including PCBs, PBDEs, and OCPs, by regulating the activation of cytochrome P450 enzymes. Previous studies examining the relationship between POPs and gene expression of molecular biomarkers indicative of contaminant exposure, have shown that expression of AhR and ARNT increases with increasing contaminant exposure in seals (Brown et al., 2014b; Lehnert et al., 2014; Lehnert et al., 2016). Our study showed similar patterns of increasing gene expression with increasing contaminant concentrations for multiple PCBs as well as PBDEs and ΣPOPs. Of the individual PCB congeners that had a relationship with AhR expression, four of them (PCB 52, 138, 153, and 180) are considered indicator PCBs with three of the congeners (PCB 138, 153, 180) being the most commonly found in marine mammal tissue (European Food Safety, 2005). PCB 52 had a positive correlation with both AhR and ARNT expression. This congener has been shown to have antagonistic interactions with these receptors and inhibit EROD activity (Aarts et

al., 1995). Brown et al. (2014b) determined a threshold value, for ΣPCBs in ringed seal blubber, of 1460 ng/lw to see an increase in AhR mRNA abundance in the liver (Brown et al., 2014b). Only one seal in this study has concentrations that fell above that threshold where a significant increase would be expected. However, if increasing expression was seen at lower concentrations than the threshold value, it may suggest that the threshold is not as accurate.

Increased expression of AhR can influence the function of metabolic enzymes such as CYP1A and contaminants can act as either inducers or inhibitors of these enzymes. The current study found a negative correlation between CYP1A expression and PCBs 105 and 180. Laboratory studies have shown that PCB 105 is an agonist of the AhR system and has shown to weakly inhibit and inactivate CYP1A (Hestermann et al., 2000; Schlezinger et al., 2006). In other mammalian species, PCB 180 has been shown to be an inducer of CYP1A in the presence of other PCBs (Van der Burght et al., 2000). Induction of CYP enzymes through alteration of the AhR pathway can potentially result in altered metabolism rate of both exogenous compounds such as contaminants and endogenous compounds including hormones (Boon et al., 1992; O'Hara and O'Shea, 2001). For some contaminants, the metabolite that would be produced through CYP enzyme activity is more toxic than the parent compound (O'Hara and O'Shea, 2001; O'Shea, 1999). Altered metabolism of endogenous compounds through induced CYP pathways can in turn affect developmental and physiological processes (Boon et al., 1992).

Contaminant related effects on thyroid hormones and pathways have been reported following exposure to PCBs, PBDEs, and OCPs. The present study found that a number of individual compounds and ΣPOPs had a positive relationship with TR-α expression in blubber. Tabuchi et al. (2006) found a positive correlation between ΣPCBs and TR-α in harbor seals

(Tabuchi et al., 2006). The study predicted that the TR-α gene may be particularly vulnerable to increasing levels of PCBs, potentially related to the apparent hypothyroidism that has been observed in individuals with high contaminants loads (Tabuchi et al., 2006). Previous studies have shown that POPs impact serum levels of both free and total T_3 and T_4 in pinnipeds (Gronnestad et al., 2018; Hall and Thomas, 2007; Villanger et al., 2013). Both T_3 and T_4 impact other hormones and enzymes related to metabolism, growth, and development through cellular activation (Hall et al., 1998; Hall et al., 2003). In order for TH mediated processes to occur, T_4 is transformed to the more bioactive T_3 at the cellular level through the action of deiodinases. Expression of DI1 was negatively related to PCB 105 concentrations, which is a dioxin-like congener that can compete with T_4 for binding sites (Soechitram et al., 2017).

Contaminants disrupt TH signaling both through reduction of TH levels as well as interaction with the thyroid receptors. PCBs have been shown to interact with thyroid receptors more when CYP1A is induced, likely due to the formation of PCB metabolites (Gauger et al., 2007; Giera et al., 2011). PCB 153 is only a slight thyroid receptor agonists, but in the presence of other compounds that induce CYP1A, increased expression of thyroid receptors is observed (Giera et al., 2011). Thyroid receptors, such as TR-α, mediate the action of TH-dependent metabolism and homeostasis in target tissues (Tabuchi et al., 2006). Of important concern for seals, THs are known to play a role in the function and maintenance of adipose tissue with TR-α being the main isoform found in blubber-like tissues (Tabuchi et al., 2006). A number of TH-regulated genes have been found in the adipose tissue of other mammals that encode for proteins involved in the metabolism of lipids (Viguerie et al., 2002). The activity of TR-α in blubber tissue demonstrates the metabolically dynamic nature of blubber, which could increase

the risk posed by contaminants on the structural and functional integrity of blubber due to alteration of metabolism within adipose tissue (Tabuchi et al., 2006).

During periods of low food intake, DI1 is preferentially increased to promote the production of reverse T_3, which helps protect individuals from the energetic burdens that can be imposed during reduced energy intake (LoPresti et al., 1991; Martinez et al., 2013). Though only one PCB showed a relationship with DI 1, there could be the potential for other contaminants and mixtures of contaminants to cause a disruption in this important metabolism pathway. Changes in both deiodination and TH receptor availability represent TH-mediated changes in metabolism (Martinez et al., 2013). Fasting or intermittently fasting northern fur seals rely heavily on blubber metabolism, mainly through lipid oxidation pathways, for energy (Martinez et al., 2013). Alterations in those pathways due to contaminant exposure could have consequences for metabolic turnover and energetics.

Studies in pups and juvenile harbor seals report endocrine and immune effects to occur at ΣPCB concentrations above 1300 ng/g lw (Mos et al., 2010). Though only one northern fur seal from this study falls above that threshold, it is important to utilize ΣPOPs since complex mixtures of contaminants have the potential to cause greater effects. ΣPOP concentrations for ten (62.5%) of the seals in this study fell above the 1300 ng/g lw threshold indicating that negative effects may be possible. The gene expression results from this study support this idea in that contaminant loads, even at low concentrations, affected gene expression. An increasing number of studies, both laboratory and field-based, are highlighting the harmful effects of complex mixtures of POPs on marine mammals (Ross, 2000). PCBs and other POPs are thought to have contributed to increases in marine mammal epizootics due to immunosuppression

caused by exposure (Ross, 2002). Mechanisms of toxicity in marine mammals are not always well understood due to the limitations of field based studies, but adverse health effects, including developmental, immunological, and reproductive, are continuing to be observed in wild populations in conjunction with contaminant exposure (Ross, 2000; Ross, 2002).

Concentrations of contaminants in Northern fur seals from St. Paul Island are largely lower than those thought to impact the function of endocrine and metabolism pathways. However, risk determination in pinnipeds based on contaminant concentration alone can be difficult due to a lack of threshold risk values for marine mammals complicated by differences due to age and sex. This study focused on sub-adult males which represent only a portion of the total population and may not fully reflect contaminant loads in adult males and females. However, results of this study do show that concentrations of contaminants measured in these seals are correlated to changes in gene expression in different physiological systems that are important in the regulation of blubber metabolism. Because northern fur seals are reliant on metabolized blubber as an energy source during periods of fasting, these data suggest that contaminants may impact animal health during critical periods.

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CHAPTER 4

QUANTITATIVE RISK ANALYSIS OF ENVIRONMENTAL EXPOSURE AND TOXIC EFFECTS OF PERSISTENT ORGANIC POLLUTANTS IN PINNIPEDS FROM THE NORTHERN HEMISPHERE

4.1 Introduction

Persistent organic pollutants (POPs) are toxic contaminants, which are hazardous to human and environmental health. POPs have long environmental half-lives and can undergo long-term atmospheric transport, allowing them to sustain their persistence once in the environment where they can bioaccumulate up the food chain (Simonich and Hites, 1995; Wania and Dugani, 2003). POP concentrations have been found at toxicologically relevant concentrations globally and they are considered ubiquitous environmental contaminants (de Wit and Muir, 2010; Wania and Mackay, 1996). Three classes of POPs, polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and polybrominated diphenyl ethers (PBDEs), have been found to accumulate throughout marine food webs due to their persistence and low metabolic degradation (O'Hara and O-Shea, 2001; O'Shea, 1999).

With increasing concentrations of persistent, lipophilic compounds in marine systems, particularly in polar regions, it is important to understand the impact on large, apex predators like marine mammals. Marine mammals are sentinel species for the systems they inhabit (Cipro et al., 2012). Their life history strategies, long life, and high trophic position make marine mammals vulnerable to biomagnification of toxicants (Desforges et al., 2013; Fair et al., 2010). Pinnipeds represent just over a quarter of the total marine mammals found globally. They are more likely to accumulate POPs due to the high lipid content in their blubber (Wolkers et al., 2006). Most POPs are taken up across the gastrointestinal tract and are eventually deposited in

blubber tissue. Pinnipeds utilize blubber as an energy source during periods of fasting that may occur during migrations, lactation, and molting (Berta, 2017; Berta et al., 2005; Riedman, 1990). Seal pups rely on the blubber stores that they develop during nursing for energy before they are ready to begin foraging on their own (Lavigne and Kovacs, 1998; Riedman, 1990). Pinnipeds inhabiting polar regions can have upwards of 30% of their body mass composed of blubber with >90% of total body lipid being in the blubber (Tanabe et al., 1981). In some species, the blubber contains 90-95% of total body POP accumulation (Tanabe et al., 1981). When those blubber stores are mobilized for energy, stored toxicants can be remobilized into circulation making them more bioavailable (Ikonomou and Addison, 2008; Wolkers et al., 2006). Blubber mobilization can also result in reconcentration of contaminants in the remaining blubber (O'Hara and O'Shea, 2001).

PCB profiles in pinniped tissue tend to be dominated by hexa- and hepta-CBs. In harbor seals and ringed seals, ΣPCBs range from 78-44,000 ng/g and 190-64,500 ng/g, respectively. The highest concentrations of ΣPCB in harbor seals were reported in the blubber and liver of adults sampled in California (Blasius and Goodmanlowe, 2008; Kajiwara et al., 2001). In ringed seals, the highest PCB concentrations are found in seals sampled in the Baltic, which were some of the original populations studied for PCB contamination (Jensen, 1966). A study examining maternal transfer of PCBs in hooded seals showed that ΣPCB concentrations in pups represented about 35% of that seen in females (Wolkers et al., 2006).

A large number of OCPs have been measured in pinniped tissues with DDT and its metabolites (DDD and DDE) being the most common. California sea lions and harbor seals have some of the highest concentrations of DDT, DDD, and DDE with ΣDDT concentrations >35,000

ng/g (Blasius and Goodmanlowe, 2008; Kajiwara et al., 2001). Chlordanes (CHL) and hexachlorocyclohexanes (HCHs) are also found in pinniped tissue with concentrations exceeding 1,000 ng/g at times (Kajiwara et al., 2001; Trukhin and Boyarova, 2013; Trukhin and Boyarova, 2020). Wolkers et al. found that female hooded seals maternally transferred up to 50% of their DDE, toxaphene, and CHL burdens to their pup (Wolkers et al., 2006).

The most common congener of PBDEs reported in seal species is BDE-47 and comprises the greatest percentage of total PBDE accumulation (Frouin et al., 2011; Ikonomou and Addison, 2008; Shaw et al., 2012; Shaw et al., 2008). Total PBDEs in harbor seals can range from 1,000-4,000 ng/g (Shaw et al., 2012; Shaw et al., 2008). Ikonomou et al. reported that upwards of 44% of ΣPBDEs were transferred from mother to pup in grey seals (Ikonomou and Addison, 2008).

All three compound classes, PCBs, OCPs, and PBDEs, have been shown to be immunotoxic, hepatotoxic, endocrine disrupters, and potentially impact reproduction. Studies suggesting a link between PCB and DDT exposure in pinnipeds and impacts on reproduction were first conducted in the 1970s and observed that ringed seals in the Baltic Sea with high concentrations of DDTs and PCBs had higher occurrences of uterine occlusions and stenosis (Helle, 1976; Helle et al., 1976). Later studies analyzing reproduction did not find a correlation between PCBs and changes in uterine pathology (Gun et al., 1992) suggesting that previous findings may have been confounded by other factors (O'Hara and O'Shea, 2001).

Endocrine-related impacts on hormones related to growth, development, and reproduction have been seen in pinniped species exposed to POPs. A decrease in reproductive hormones has been observed with increasing concentrations of PCBs (Troisi et al., 2020), while

DDT and DDE can potentially disrupt estrogen receptor signaling pathways (Yoshinouchi et al., 2019). Effects on thyroid hormones have been reported for PCBs, OCPs, and PBDEs in pinnipeds. Thyroid hormones have been shown to be affected in both free and total forms as well as the relationship between T_4 and T_3 (Gronnestad et al., 2018; Hall and Thomas, 2007; Sormo et al., 2005; Villanger et al., 2013). There is also evidence that hydroxylated metabolites, of PCBs and PBDEs, may interact with thyroid hormone receptors, which can alter transcription activity and gene expression, thus influencing the regulation of genes that are crucial at different developmental stages (de Wit, 2002; Tabuchi et al., 2006).

Contaminant-induced immunosuppression is a growing concern due to the potential for increased susceptibility to disease. A series of studies done on semi-captive harbor seals demonstrated decreased immune function in seals fed contaminated fish (de Swart, 1995; De Swart et al., 1996; Ross et al., 1995; Ross et al., 1996). The studies found that T lymphocytes were impacted in multiple ways including decreased mitogen and antigen induced proliferation, decreased mixed lymphocyte reaction, and delayed skin sensitivity response in seals fed a contaminated diet (de Swart, 1995; Ross et al., 1995). Natural killer cell activity was also significantly decreased and that neutrophil counts were significantly increased in contaminated seals (de Swart, 1995; Ross et al., 1996).

Pinnipeds were some of the first marine mammals to have PCBs and other POPs detected in their tissues (Jensen, 1966). Since the discovery of persistent contaminants, concentrations of PCBs, OCPs, and PBDEs have been reported in thousands of samples from pinnipeds across the globe. Since 2000, over 100 studies have reported PCB, OCP, and/or PBDE concentrations in at least 15 species of pinnipeds with fewer than half of those examining

health effects. Understanding potential adverse effect concentrations as a component of a quantitative risk assessment is critical to the conservation of these species (Hutchinson and Simmonds, 1994; Kannan et al., 2000).

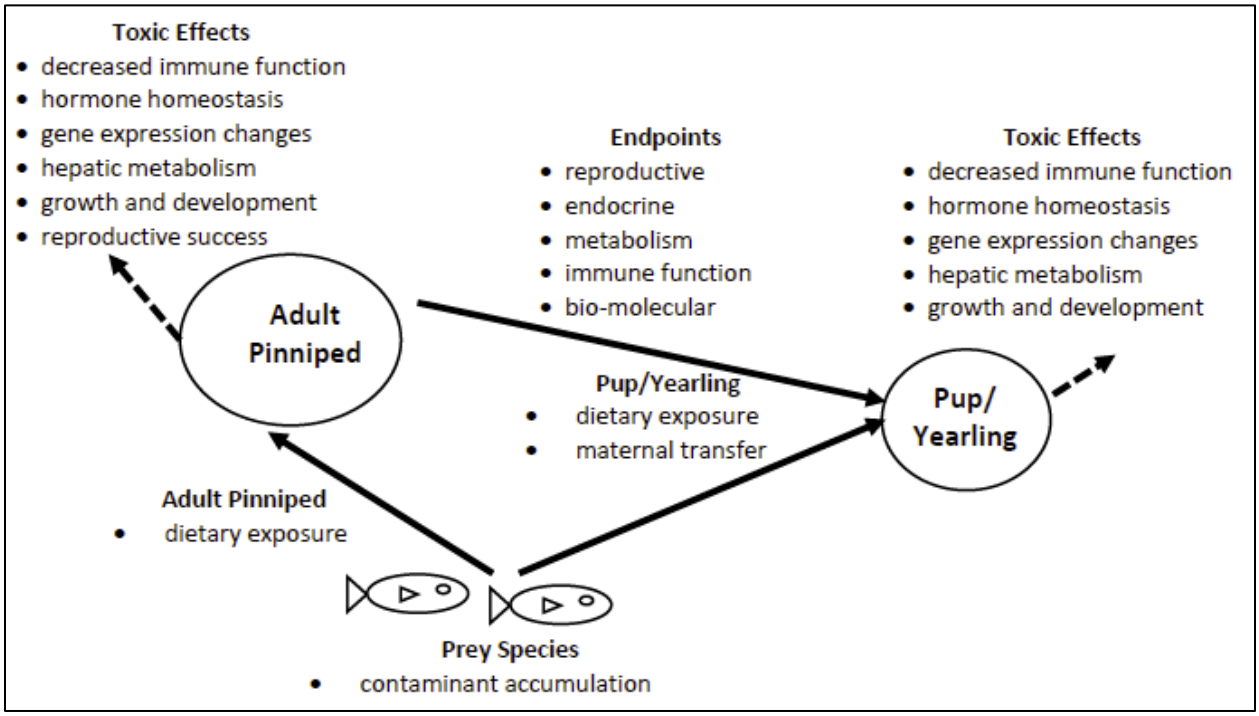

Figure 4.1: **Exposure pathways (solid arrows), physiological endpoints, and potential toxic effects (dashed lines) for pups/yearlings and adult pinnipeds exposed to persistent organic pollutants.**

The goal of this analysis is to assess POP exposure in wild pinniped populations, estimate toxic effect threshold concentrations, and determine potential risk posed by POPs to pinniped health (Figure 1). This risk assessment is focused on PCBs, PBDEs, and OCPs, specifically DDT and its metabolites, due to their known accumulation in pinnipeds and potential for toxic effects. Pinniped species found in the northern hemisphere, mainly in polar and temperate regions, will be the focus due to the availability of data and the widespread distribution of some of the species. Estimated exposure concentrations were determined from literature-reported values and toxicity effect concentrations were calculated as EC_{20} and EC_{50} based on a meta-analysis of

existing toxicity data from pinnipeds and related species. Here, we show that POPs are accumulating in pinniped species across the northern hemisphere and that concentrations of POPs in some wild pinnipeds exceed our estimated toxic effect thresholds (Figure 4.1).

4.2 Methods

4.2.1 Literature Search

Literature searches were conducted using Web of Science and Google Scholar search engines using the following search terms: “pinnipeds PCBs”, “pinnipeds PBDEs”, “pinnipeds organochlorine”, “pinnipeds DDT”, “pinnipeds contaminants”, “pinnipeds chlorinated pesticides”, and all were repeated with the term pinniped replaced with seal(s). Papers chosen for the assessment fit within a set of conditions: 1. Published within the last 20 years, 2. Studied pinnipeds from the northern hemisphere, allowing for assessment of both phocid and otariid species with the opportunity to assess some species across multiple locations, and 3. Contain data on at least one of the classes of compounds of interest. For papers that studied endpoint(s) of exposure, the publication year condition was removed.

4.2.2 Data Collection

Papers that met the conditions for inclusion then had all concentration data collected and placed into a spreadsheet. The following data were collected from each paper when available: source, species, location of sampling, decade and years sampled, demographic factors (age class, sex, tissue), tissue analyzed, and contaminant data (contaminant group, congener/compound, mean concentration, range, unit including lw/ww). Similar demographic data was pulled from effects papers as well as which endpoint was measured and a brief

description of the findings. The toxic effects papers were divided into the following categories: endocrine, immune, hepatic, bio-molecular, and reproductive.

4.2.3 Data Analysis

Dose response data was compiled into a spreadsheet and response values were converted to percent control due to differences in endpoints, measurement methods, species used, etc. This allowed us to normalize response data and classify it by physiological system (e.g. immune, endocrine, etc.). EC_{20} and EC_{50} values, with upper and lower confidence intervals, were calculated in JMP (SAS Institute) relative to control using an inverse prediction on a logistic 3P fit curve (Figure 4.2) (SAS Institute, 1989-2007). Any confidence intervals that came back with a lower estimate below zero were not included in further analyses. If the 95% confidence intervals for the EC_{20} and EC_{50} overlapped (in other words, they were not different), then only EC_{50} was used for analysis. If there was no overlap between confidence intervals, then both the EC_{20} and EC_{50} were retained for analysis.

Toxicity effect levels were categorized by system (e.g. immune, endocrine), tissue, and contaminant analyzed. Toxicity reference values (TRV) were then determined by calculating a mean toxicity effect level for each system-tissue-contaminant combination. For system-tissue-contaminant combinations where there was only one toxicity effect level, then that was used as the TRV. Hazard quotients (HQ) were calculated for each of the tissue-contaminant combinations that were represented by toxicity effect levels using the ratio of the mean environmental concentration for the tissue-contaminant combination and the matching toxicity reference value for each given system. A HQ greater than 1 indicates a potential risk, moderate (1.1-10) or severe (>10), and HQs less than 1 indicate a low to negligible risk (Lemly, 1996).

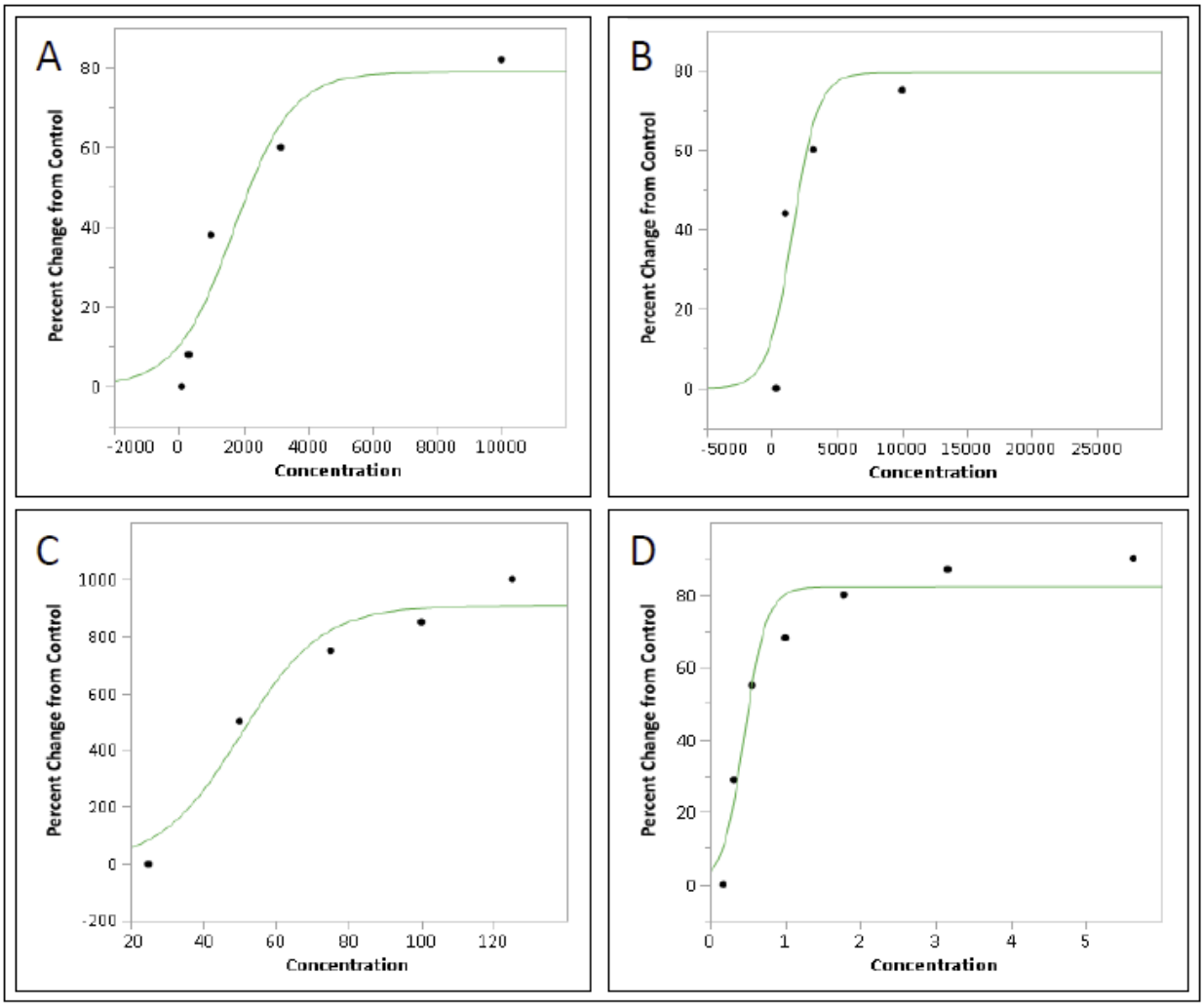

Figure 4.2: Example dose response curves for ΣPCBs in blubber (ng/g) and A. endocrine, B. immune, C. hepatic metabolism, and D. bio-molecular endpoints.

4.3 Results

4.3.1 Environmental Exposure

A total of 101 papers met the conditions to be included in the exposure estimates. These papers represent concentration data from 14 species (3 otariids and 11 phocids) sampled from 25 locations across the northern hemisphere. Harbor and ringed seals were the most common species (27% and 31% of papers, respectively) followed by grey seals and northern elephant seals. Alaska, Canada, Norway, California, and Greenland represented the locations

where the most studies have been conducted (15%, 23%, 10%, 14%, and 8% of papers, respectively). There were 10 contaminant groups represented with PCBs, DDTs, and PBDEs being the top three studied (77%, 60%, and 46% of papers, respectively). When assessing tissue analyzed, blubber was the most commonly analyzed (in 84% of studies) followed by blood/serum, liver, and milk (24%, 17%, and 9% of papers, respectively).

PCBs were not only the most studied compounds, but they also represent some of the highest concentrations reported in pinnipeds. Across all species, the mean ΣPCB concentrations in blubber, liver, and blood were 10,212.94 ng/g (range: 0-951,500); 36,885.95 ng/g (range: 0-599,500); and 1,461.54 ng/g (0-33,000), respectively. These high concentrations in blubber and liver tissues are driven by California sea lions, harbor seals, and northern elephant seals sampled in California (Blasius and Goodmanlowe, 2008; Greig et al., 2011; Kajiwara et al., 2001), several phocids (harbor, hooded, and grey seals) sampled in Quebec (Hobbs et al., 2002), and grey and ringed seals from the Baltic Sea region (Nyman et al., 2003; Nyman et al., 2002). Ringed and harbor seals, however, also had some of the lowest reported concentrations but from different parts of their range (Helm et al., 2002; Wang et al., 2012). There were five PCB congeners that were measured most frequently across all tissue types: CB 105, 118, 138, 153, and 180. These congeners are known to be the most prevalent in environmental samples. PCBs were found in locations across the entire northern hemisphere with some areas being hotspots. California, especially central and southern coastal areas, represented the highest concentrations for a number of species (Blasius and Goodmanlowe, 2008; Greig et al., 2011; Kajiwara et al., 2001). The Baltic Sea is known to be an area with high concentrations of PCBs found in seal tissue which was reflected in our results. Some areas of Canada including Quebec

were hotspots for specific species but also had some of the concentrations found on the lower end of the range. Hydroxylated PCB metabolite concentrations followed a similar pattern to the parent compounds. When measured, OH-PCBs were most often measured in serum/blood to assess at circulating concentrations or in the liver. Concentrations in the liver were much higher than the circulating concentrations suggesting that metabolites are being stored in liver tissue.

Though considered an emerging contaminant, our results demonstrate that PBDEs are accumulating in seal tissue across the northern hemisphere. The highest PBDE concentrations were observed in blubber tissue where the mean ΣPBDE was 294.02 ng/g (range: 0-23,510). The five most common environmental PBDEs, BDE 47, 99, 100, 153, and 154, were the most often observed in pinnipeds with BDE 28 and 183 also commonly occurring. Pinnipeds sampled in California had the highest concentrations of PBDEs (Blasius and Goodmanlowe, 2008; Greig et al., 2011; Kajiwara et al., 2001), with harbor seals sampled along the northwest Atlantic coast and Steller sea lions exhibiting higher accumulation (Alava et al., 2012; Shaw et al., 2008). Methoxylated BDEs were found in higher concentrations than hydroxylated BDEs, but there are very few studies that measured hydroxylated BDE accumulation. The MeOBDE concentrations were mostly measured in blubber tissue with the highest concentrations seen in ringed seals sampled in the Arctic (Kelly et al., 2008b; Letcher et al., 2009). In most cases, however, PBDEs were found at concentrations that were at least an order of magnitude lower than PCB concentrations.

Over 20 compounds were measured that are classified broadly as OCPs. DDT and its metabolites were the most commonly measured followed by HCHs and CHLs. Other major OCPs including toxaphenes, dieldrin, aldrin, endrins, endosulfans, heptachlors, and

heptachlorobenzene (HCB) were also measured in a variety of pinniped tissues. ΣDDT concentrations in pinniped tissues represented the highest concentrations seen across all compounds. The mean ΣDDT concentrations in blubber, liver, blood, and milk were 32,202.79 ng/g (range: 0-4,030,610); 32,870.37 ng/g (range: 0-702,300); 3,682.47 ng/g (range: 0.48-74,000); and 466.59 ng/g (range: 1.5-1,918), respectively. DDE made up the majority of the ΣDDTs which is consistent with other environmental samples (O'Hara and O'Shea, 2001; O'Shea, 1999). DDT was found in pinniped tissue throughout the northern hemisphere with California, Japan, Baltic, and areas in the northwest Atlantic Ocean being hotspots. Similar to what was reported in PCBs, species such as harbor and ringed seals represented both the high and low ends of the spectrum dependent on the locations of the populations that were sampled.

HCH concentrations were highest in the blubber, 522.56 ng/g, followed by liver, blood, and milk. The majority of ΣHCHs were α-HCH and γ-HCH except for in blood where β-HCH was the most dominant. Spotted seals sampled in Japan had the highest HCH concentrations with California sea lions and northern elephant seals also having elevated concentrations. CHLs were measured less frequently but are found at higher concentrations than HCHs. The mean ΣCHL concentrations in blubber, liver, blood, and milk were 2,190.07 ng/g (range: 0-670,370); 2,304.44 ng/g (range: 0-23,920); 132.45 ng/g (range: 0.3-609.23); and 210.93 ng/g (range: 0.45-1,650), respectively. California sea lion blubber and liver tissue had significantly higher CHLs than any other species, 18,910.8 ng/g and 11,115.56 ng/g, respectively (Blasius and Goodmanlowe, 2008; Kajiwara et al., 2001). The next highest concentrations were reported in harbor and hooded seals sampled in the northwestern Atlantic (Hobbs et al., 2002; Wolkers et al., 2006). When assessing the remaining OCPs, HCB was the most commonly measured in

pinniped tissue (blubber, blood, serum, liver, and milk) with mean HCB concentrations in blubber of 20.67 ng/g (range: 0-440). The other OCPs were found mostly in blubber tissue with sum heptachlors being the highest and aldrin the lowest. Overall ΣOCP concentrations (not including DDT in most cases) in blubber had a mean of 1,035.67 ng/g (range: 2.88-11,000). Similar to what has been reported with the other contaminants, California sea lions had the highest accumulation compared to other pinniped species.

4.3.2 Toxic Effects

For papers studying toxic effects, 65 papers were found of which 32 had data that met the inclusion criteria. Toxic effects that fell into the categories of immune and endocrine were the most commonly studied (56% and 22% of papers, respectively). An EC_{20}, EC_{50}, or both, relative to control, were calculated for 24 of the toxic effect papers. Toxicity effect levels were calculated for ΣPCBs, six individual PCB congeners (CB 118, 138, 149, 153, 156, and 175), OH-PCBs, ΣPBDEs, and three individual PBDEs (BDE 47, 99, and 153) (Table 4.1). Of the toxicity effect levels calculated, a majority represented studies analyzing lymphocyte proliferation and thyroid hormone levels. There were no papers or data sets that met our criteria for any of the PBDE metabolites or OCP compounds or for reproductive endpoints, therefore no toxicity effect levels or TRVs were calculated. A total of 38 HQ were calculated representing 24 tissue-contaminant combinations and four toxic effects endpoints: immune, endocrine, hepatic, and bio-molecular (Table 4.1).

Table 4.1: Hazard quotients calculated for the different tissue-contaminant combinations including the mean and range of tissue concentrations, toxicity effect level used to determine the TRV, physiological system, toxicity reference value, and hazard quotient.

Tissue	Compound	Mean Concentration (ng/g)	Concentration Range (ng/g)	Toxic Effect Level/System	Toxicity Reference Value (ng/g)	Hazard Quotient
Serum	3-OH-CB138	0.12	0.02-0.53	EC_{20}/Endocrine	0.00002	6,500
Serum	4-OH-CB107	1.02	0.18-6.44	EC_{50}/Endocrine	0.003	383.77
Liver	OH-O5CBs	7.15	0-123	EC_{50}/Endocrine	1.02	7.04
Liver	OH-O6CBs	1.33	0-218	EC_{50}/Endocrine	1.02	1.31
Liver	OH-O7CBs	2.74	0-149	EC_{50}/Endocrine	1.02	2.7
Liver	OH-O8CBs	0.08	0.002-0.38	EC_{50}/Endocrine	1.02	0.08
Blubber	PCB 118	151.92	0-3,000	EC_{50}/Immune	59.37	2.56
Blood	PCB 118	12.02	0.01-74.33	EC_{50}/Endocrine	1.51	7.96
Blubber	PCB 138	1,904.27	0.10-23,800	EC_{50}/Immune	12,380	0.15
Blubber	PCB 149	121.95	4.5-2,161.43	EC_{50}/Immune	5.9	20.66
Blubber	PCB 153	2,964.50	0.19-72,100	EC_{50}/Immune	19,780	0.15
Blubber	PCB 156	45.9	0-615.22	EC_{50}/Immune	35.5	1.29
Blubber	PCB 157	16.37	0.01-130	EC_{50}/Immune	15.85	1.03
Blubber	PCB 180	943.6	0-21,900	EC_{20}/Endocrine	45.52	20.29
				EC_{50}/Immune	17,670	0.05
Liver	Sum OH-PCB	19.95	0.09-69.3	EC_{50}/Endocrine	4.67	4.28
Serum	Sum OH-PCB	1.38	0.34-4.13	EC_{50}/Immune	0.35	3.98
Blubber	Sum PCB	10,212.94	0-951,500	EC_{50}/Immune	2,358.41	4.33
				EC_{50}/Bio-molecular	528.39	19.33
				EC_{50}/Hepatic	0.019	539,796
				EC_{20}/Immune	2,774.49	3.68
				EC_{50}/Endocrine	15,675	0.65

Tissue	Compound	Mean Concentration (ng/g)	Concentration Range (ng/g)	Toxic Effect Level/System	Toxicity Reference Value (ng/g)	Hazard Quotient
				EC_{50}/Endocrine	2.21	4,619.76
Blood	Sum PCB	1,461.54	0-33,000	EC_{50}/Endocrine	13.54	107.94
				EC_{50}/Immune	7.75	188.59
Liver	Sum PCB	36,885.95	0-599,500	EC_{50}/Hepatic	1,623.33	22.72
Blood	BDE 47	0.1	0.003-0.44	EC_{50}/Hepatic	0.49	0.2
				EC_{20}/Immune	3.99	0.03
				EC_{20}/Endocrine	3.27	0.03
Blood	BDE 99	0.04	0.01-0.14	EC_{50}/Hepatic	0.49	0.08
				EC_{20}/Immune	3.99	0.01
				EC_{20}/Endocrine	3.27	0.01
Blood	BDE 153	0.04	0.004-0.16	EC_{50}/Hepatic	0.49	0.08
				EC_{20}/Immune	3.99	0.01
				EC_{20}/Endocrine	3.27	0.01
Blubber	Sum PBDE	294.02	0-23,510	EC_{50}/Immune	8.38	35.11
Serum	BDE 99	13.46	0-7.95	EC_{50}/Endocrine	0.13	102.13

4.3.2.1 Immune Function

Contaminant effects on immune function have been well-studied in both laboratory and field studies. Our results found that immune endpoints were the most commonly reported in pinniped studies with impacts of exposure on immune responses, such as lymphocyte proliferation, vitamin A levels, and phagocytosis, measured most often. Toxicity effect levels for immune endpoints were calculated for ΣPCBs in blubber and blood, seven individual PCBs in blubber, ΣOH-PCBs in serum, ΣPBDEs in blubber, and three individual PBDEs in cells.

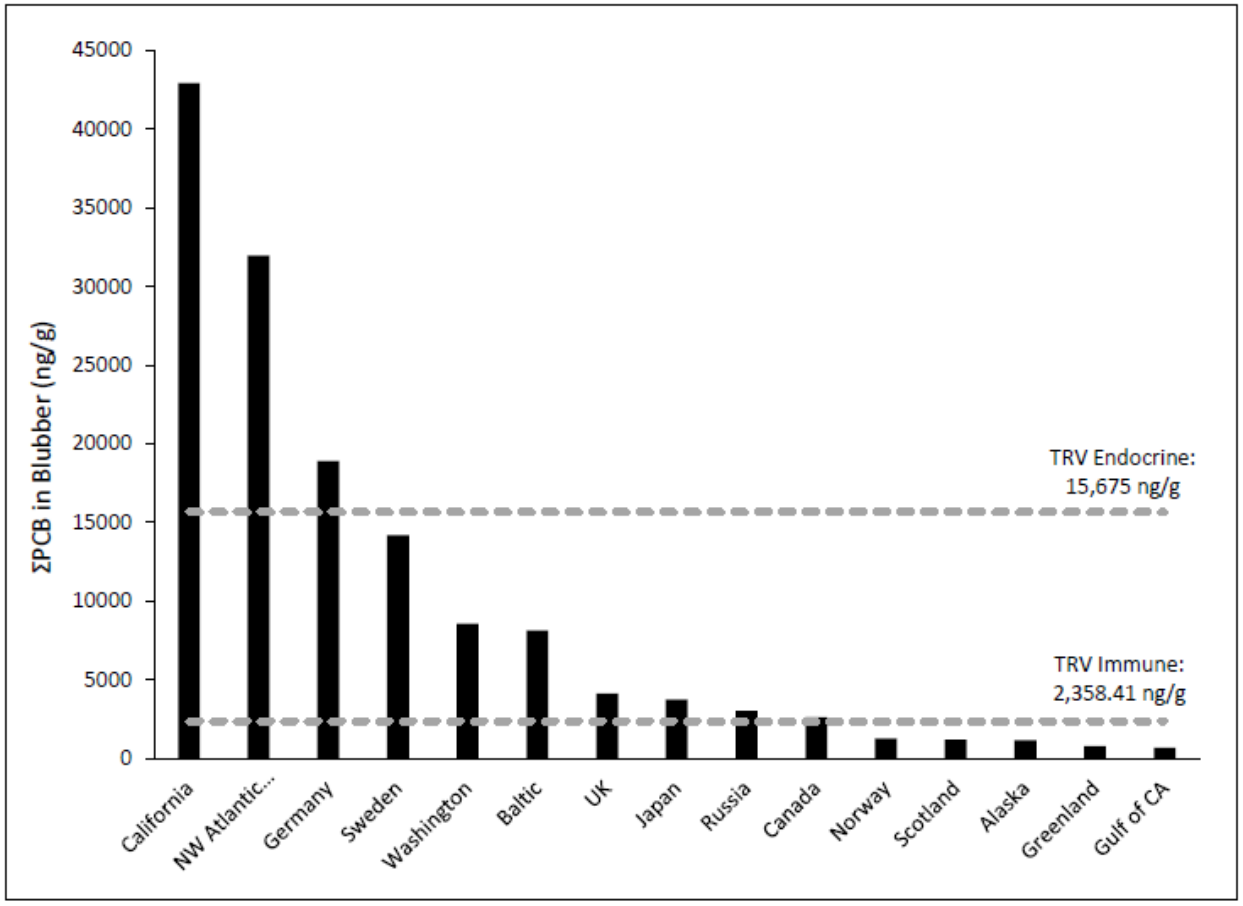

Figure 4.3: Mean ΣPCBs (ng/g) in blubber tissue from fifteen locations compared to the calculated TRVs for endocrine and immune endpoints.

The calculated toxicity effect concentrations for immune endpoints compared to ΣPCBs in blubber ranged from 0.03-12,470 ng/g and in blood, only one toxicity effect level was

calculated at 7.75 ng/g. For individual PCB congeners, toxicity effect levels ranged from 2.96 ng/g (PCB 149) to 19,780 ng/g (PCB 153). Only one toxicity effect level was calculated for ΣOH-PCBs at 0.35 ng/g. The toxicity effect level for ΣPBDEs was 8.38 ng/g, and for the individual PBDE congeners, the calculated toxicity effect levels ranged from 2.86 ng/g (BDE 47) to 5.33 ng/g (BDE 99). TRVs for immune endpoints compared to individual congeners and ΣPCBs in blubber were found to be from <1 up to 2,358.41 ng/g for EC_{50} and 2,774.49 ng/g for EC_{20}, when that was the only toxicity effect level available (Figure 4.3). The TRV for immune endpoints and individual PBDE congeners was 3.99 ng/g for EC_{20}.

4.3.2.2 Endocrine Effects

This study found that effects on circulating levels of both thyroid hormones are the most often studied to assess the impact of contaminant accumulation. For endocrine endpoints, toxicity effect levels were calculated for ΣPCBs in blubber and blood, one PCB congener in blubber, one PCB congener in blood, OH-PCBs in liver, two individual OH-PCBs in plasma, and three PBDE congeners in cells and serum. Toxicity effect levels for endocrine related endpoints for ΣPCBs in blubber ranged from 2.21-19,850 ng/g and in blood, the toxicity effect level was 13.54 ng/g. For PCB congeners, toxicity effect levels were 27.03 ng/g and 66 ng/g for PCB 180 in blubber and 1.51 ng/g for PCB 118 in blood. OH-PCB toxicity effect levels ranged from 0.25-4.97 ng/g. The two individual OH-PCBs had toxicity effect levels of 0.000018 ng/g and 0.003 ng/g. PBDE congener toxicity effect levels ranged from 1.55 ng/g (BDE 47) to 4.4 ng/g (BDE 99) and 0.13 ng/g for BDE 99 in plasma. Effects of OH-PCBs were more prevalent when studying endocrine endpoints compared to parent PCBs. The calculated TRVs for OH-PCBs, 1.02 ng/g and 4.67 ng/g, varied greatly from the PCB TRV 15,675 ng/g, with the variation being due to tissue

and contaminant of interest (Figure 4.3). The calculated TRV for PBDEs was 3.27 ng/g.

4.3.2.3 Hepatic Metabolism and Bio-Molecular Effects

For hepatic endpoints, toxicity effect levels were calculated for ΣPCBs in blubber and liver and three individual PBDE congeners in cells. The toxicity effect levels for hepatic endpoints for ΣPCBs in liver ranged from 380-2,510 ng/g and was 0.02 ng/g in blubber. For PBDE congeners, toxicity effect levels ranged from 0.35 ng/g (BDE 47) to 0.62 ng/g (BDE 153). The TRV for ΣPCBs in liver was 1623.33 ng/g and 0.44 ng/g for PBDE congeners. Toxicity effect levels for bio-molecular endpoints ranged from 395.16-660 ng/g for ΣPCBs in blubber. The calculated TRV for ΣPCBs in blubber was 528.39 ng/g.

4.4 Discussion

Pinnipeds are exposed to a complex mixture of environmental contaminants that occur at concentrations that, based on this analysis, may cause adverse effects at both the individual and population level. Overall POPs preferentially accumulate in the blubber and liver tissues as demonstrated by the high concentrations in those tissues. Though concentrations in blood and serum were not as high, contaminant concentrations in those tissues can help to understand which compounds are more likely to be found in circulation where they have the potential to be more bioavailable than those being stored in blubber. Concentrations of contaminants seen in wild pinnipeds do exceed the TRVs for both immune and endocrine endpoints. Impacts on these two physiological systems could have potential long-term effects on both individuals as well as larger populations. Long-term impacts of contaminant exposure coupled with other

stressors, such as climate change and habitat disturbance, could affect the fitness and survival of pinniped populations.

4.4.1 Exposure Assessment

4.4.1.1 Spatial Trends

Across all contaminant groups, there are hotspots in the northern hemisphere where contaminant concentrations are higher than other areas and represent the highest concentrations reported. Central and southern California represented the highest measured POP concentrations across multiple contaminant groups. The three species studied in this area, California sea lions, harbor seals, and northern elephant seals, have varying foraging patterns, prey, and weaning strategies that can result in exposure to contaminants from a variety of sources. These areas of the California coast have agricultural and industrial inputs of contaminants including the highly contaminated southern California Bight (Blasius and Goodmanlowe, 2008). California sea lions forage throughout the Bight and are known to migrate up and down the coastline, which likely accounts for high accumulation of contaminants (Blasius and Goodmanlowe, 2008; Greig et al., 2011; Kajiwara et al., 2001). Pinnipeds sampled in the waters in and around Japan also had high contaminant loads, particularly DDT (Kajiwara et al., 2004). Inputs from agricultural sources from multiple countries likely lead to the increased concentrations of observed DDTs in northern fur seals and spotted seals sampled in these water (Trukhin and Boyarova, 2013; Trukhin and Boyarova, 2020). Increased concentrations of PCBs are attributed to the continued release from products containing PCBs that are currently being broken down or degrading. For some species, the northwest Atlantic coast (Canada and the United States) represents a hotspot for increased

concentrations of PCBs, PBDEs, and DDTs. Some areas of the Arctic from Russia and Alaska to the Canadian Arctic and Greenland have patches of increased contaminant accumulation. In these areas, the source of contaminants is from long-term atmospheric transport and deposition. Increasing concentrations of POPs in the tissue of pinnipeds inhabiting these high latitude areas can indicate a growing environmental problem since unlike other areas of high contamination there are no major point source inputs (de Wit et al., 2006; Ikonomou et al., 2002).

4.4.1.2 Temporal Trends

The papers utilized in this analysis were all published within the last 20 years. Across the sampling periods, there are temporal trends that can be observed for contaminants in multiple locations. In California sea lions, PCB concentrations were highest in the 1990s and have decreased with some evidence of leveling off (Blasius and Goodmanlowe, 2008; Kajiwara et al., 2001). Northern elephant seals and harbor seals sampled in that area have a similar pattern. Ringed seal blubber sampled from across its range, shows a decrease in PCB concentrations with a leveling off occurring in conjunction with when PCB production was stopped/slowed. In Sweden, ringed seal blubber has had a consistent decline in PCBs since the 1970s, with the rate of decline slowing in recent years (Bjurlid et al., 2018). Though there is a decrease in PCB concentrations across the northern hemisphere, often thought to be tied to bans, in most areas the current concentrations are staying more consistent due to continued PCB inputs from the degradation of products containing PCBs (Blasius and Goodmanlowe, 2008; Kajiwara et al., 2001).

Production of PBDEs peaked in the late 1990s and early 2000s, which is reflected in

measured environmental concentrations (Shaw et al., 2008; Shaw and Kannan, 2009). Across most of the northern hemisphere, PBDE concentrations in pinniped tissues increased during the 1980s and 1990s with peak concentrations occurring in the late 1990s and 2000s. PBDE concentrations in grey seals sampled in Scotland decreased significantly from the 2000s to the 2010s (Robinson et al., 2018; Vanden Berghe et al., 2012).

DDTs have more temporal variability that, in a number of cases, can be tied to location. In some regions, DDT concentrations have remained constant over the last few decades with a slightly declining trend. In Japan, for example, DDT concentrations in northern fur seals decreased from concentrations measured in the 1970s but spotted seals sampled in 2010 had very high concentrations of DDT (Kajiwara et al., 2004; Trukhin and Boyarova, 2013; Trukhin and Boyarova, 2020). DDT concentrations in ringed seals sampled in Canada declined from the 1970s to the 1980s followed by an increase in the 1990s and another decrease in the following decades. Other OCPs, like CHLs and HCHs, have similar temporal patterns to that of DDT with some peaks in concentrations potentially due to point source inputs from agricultural areas. Even though a number of POPs have now been banned or decreased in production, they are still being input into the environment from product disposal or production in certain countries.

4.4.1.3 Exposure Uncertainties

Marine mammals as a group have unique physiological and ecological demands. For pinnipeds, those demands vary across species but also across populations of a single species based on location, life stage, and prey preference. Variations in blubber usage at different life stages and different times of the year can affect contaminant loads. How exposure data is collected, such as location, tissue, and species, and how the data is then analyzed (e.g. the

number and types of compounds) can impact the interpretation of exposure data and the comparison between studies. Our study focused on three major groups, PCBs, PBDEs, and OCPs, but other contaminants and heavy metals, such as mercury, are present in pinnipeds. Mercury has been measured in pinniped tissues and is known to have a variety of toxic effects even at low levels of exposure (Beckmen et al., 2002). The potential for effects to occur due to the interaction between mercury and other contaminants, like POPs, should be a concern when assessing toxic effects in pinnipeds, especially in young animals (Beckmen et al., 2002).

4.4.2 Toxic Effects

Previous studies assessing the risk of POPs to pinnipeds have focused on narrow demographic or contaminant groups and have often presented TRVs based on dietary intake or PCB toxic equivalency values (Kannan et al., 2000; Mos et al., 2010). The present study calculated TRV by physiological system to get an understanding of the risk for the different endpoints within a system. Future analyses where endpoint TRV are broken up further within the physiological system will be useful in determining the overall risk of exposure. This assessment combined data from a range of pinniped contaminant studies but future assessment should take into account the different physiological, ecological, and demographic factors that can affect body burdens for individual species as well as groups of similarly related species. Previous studies have shown that immune and endocrine systems can be affected at PCB concentrations above 1,300 ng/g, in juveniles and pups, which is lower than the TRV estimates from this study (Mos et al., 2010). Over 50% of seals had blubber PCB concentrations that fell above that 1,300 ng/g threshold value, but that portion may be skewed towards adult seals which are likely to have the higher body burdens. The discrepancies in these TRV values

demonstrate that future studies should take other factors including sex and age class into account.

4.4.2.1 Immune Function

Forty percent of seals had blubber PCB concentrations that fell above the calculated TRV for immune effects (lymphocyte proliferation). This would indicate that not all seals sampled for PCBs would be at a high risk for immune effects, but there are populations where toxic effects are likely to occur even at lower concentrations. The HQs for immune endpoints varied highly with three falling below 1, indicating a negligible/low risk, six HQs were in the range for a moderate effect, and two were above 10 indicating a potentially severe risk. When analyzing the HQs for ΣPCBs in blubber, the mean ΣPCB concentrations in blubber are being driven by subsets of pinnipeds, mainly from California, with very high concentrations. Only 35% of papers examining ΣPCBs in blubber in non-California pinnipeds had mean ΣPCB concentrations that exceeded the TRV. This indicates that there are still multiple populations of pinnipeds at a high risk for toxic effects. Populations in those studies that fell below the TRV may still be at risk when other compounds are taken into account along with PCBs. Hazard quotients for Individual PBDE congeners did not indicate significant risk to immune function, but the HQ for ΣPBDEs in blubber was greater than 10 suggesting a severe risk. Studies have shown that increased accumulation of toxic contaminants can affect immune system function, which in turn could increase susceptibility to disease (De Swart et al., 1996; Desforges et al., 2016; Mos et al., 2006; Ross, 2002; Van Loveren et al., 2000). Both PCBs and DDTs have been suspected to cause increased immunological disorders in many species of pinnipeds (Kannan et al., 2000). Studies done on pinniped species inhabiting the Baltic and Wadden seas have shown that increased

disease prevalence and decreased immune function have been associated with high concentrations of these persistent compounds (De Swart et al., 1996; Olsson et al., 1994; Reijnders, 1986). Since 1991, there have been seven unusual mortality events (UMEs) declared for pinnipeds in both the Atlantic and Pacific where the cause was determined to be infectious disease (OPR, 2020). Immunosuppression in pinnipeds has been hypothesized to be initially caused by increasing concentrations of toxic contaminants, such as PCBs, PBDEs, and OCPs, that cause them to be more susceptible to diseases (Kannan et al., 2000; Lahvis et al., 1995).

4.4.2.2 Endocrine Effects

The endocrine system is key for the growth and development of pinnipeds as well as in the production of reproductive hormones. Levels of endogenous compounds, like thyroid hormones, vary with age with the impact of contaminants potentially varying as well. Only 16% of seals had PCB in blubber concentrations that exceeded the estimated TRV for thyroid hormone level suggesting that most seals sampled would not be at a high risk. Thyroid hormones are affected by the presence of contaminants, especially the metabolized forms, which have a high binding affinity for both carrier proteins and cellular receptors (Darnerud et al., 2001; de Wit, 2002; Hallgren et al., 2001). Thyroid hormones affect other hormones and enzymes mostly related to metabolism, growth, and development. When assessing ΣOH-PCB and OH-PCB groups (by chlorination level) in the liver, only one HQ falls below 1, while four are within the moderate risk range. Individual OH-PCBs in serum had HQ that potentially indicate a severe risk to thyroid hormone levels even at low concentrations. PCBs in blubber tissue and blood all had HQs that indicate either a moderate or severe risk to endocrine function. For endocrine effects, only 8% of papers examining ΣPCBs in non-California and non-Baltic

pinnipeds exceeded the TRV for circulating thyroid hormone levels. This would suggest that most pinnipeds do not have concentrations of PCBs that pose a significant risk. The HQ for circulating concentrations of one PBDE congener (BDE 99) was greater than ten indicating a potentially severe risk for effects on thyroid hormone.

Interaction with thyroid cellular receptors and increased expression of genes controlled by those receptors has been observed in pinnipeds sampled from high contamination areas and with increasing PCB and DDT exposure (Routti et al., 2010; Tabuchi et al., 2006). A number of studies have shown that effects of contaminants can be greater for T_3 compared to T_4 which can be problematic since T_3 is the more bioactive hormone (Chiba et al., 2001; Gronnestad et al., 2018; Hall and Thomas, 2007; Sormo et al., 2003; Villanger et al., 2013). Some of the effects of contaminants on T_3 are thought to be due to impacts at the cellular level. Contaminants can bind to the deiodinases, which impact the production of T_3, or interfere with carrier proteins (Schuur et al., 1998). Maintenance of thyroid homeostasis in pinnipeds is crucial during times of development and fasting because blubber metabolism is highly regulated by the endocrine system. Pinnipeds feeding in areas of known contamination have significantly lower levels of some reproductive hormones, including estradiol and esterone, which negatively correlate with PCB concentrations in female seals (Troisi et al., 2020). Contaminant activation of estrogen receptors in mammals including seals has a dose-dependent relationship with OCPs, especially DDT and DDE, at concentrations found in wild populations of pinnipeds (Yoshinouchi et al., 2019).

4.4.2.3 Hepatic Metabolism and Bio-Molecular Effects

Bio-molecular endpoints, such as changes in gene expression or cellular receptor

pathways, can be used to help better understand potential impacts at the cellular level. Changes in these bio-molecular endpoints can serve as early indicators of higher-level effects due to contaminant exposure (Tabuchi et al., 2006). After uptake, metabolic pathways in the liver convert parent POPs into more polar metabolites for excretion. Multiple studies have shown dose-dependent increases in the activity of cytochrome P450 enzymes and other phase 1 enzymes (EROD and MROD) after exposure to PCBs and OCPs (Routti et al., 2008; Troisi and Mason, 2000; Wolkers et al., 2009; Wolkers et al., 2002). POPs can act as CYP450 enzyme inducers, inhibitors, or substrates. In pinniped tissue, multiple enzymes are present meaning that the CYP450 system can metabolize many different compounds at once (O'Hara and O'Shea, 2001; O'Shea, 1999). The produced metabolites can be more toxic or have different mechanisms than the parent compound (Boon et al., 1994; Boon et al., 1992). The induction of CYP450 enzymes can also potentially result in altered metabolism of endogenous compounds, such as hormones, which can in turn affect developmental and physiological processes (Boon et al., 1992; O'Hara and O'Shea, 2001). The HQ determined for both blubber and liver ΣPCBs fell above 10 suggesting a severe risk for induction of hepatic metabolic pathways in the presence of PCBs. The HQs for Individual PBDE congeners did not indicate significant risk.

4.4.3 Conclusions

The risk of toxic effects due to contaminant exposure in pinnipeds can be highly variable and influenced by multiple factors including location, sampling period, and tissue type. Hazard quotients for POPs in pinnipeds ranged from low to severe depending on contaminant type and location of the population. Overall, PCB accumulation posed the greatest or most frequent hazard to marine pinnipeds while PBDEs were the lowest/least frequent. An overall lack of

toxicity data made the assessment of OCPs difficult. Though this study indicates that certain pockets of pinnipeds are accumulating contaminants at concentrations high enough to have toxic effects, it is increasingly important to understand this complex relationship even at lower concentrations. The relationship between contaminants and toxic effects of exposure, immune suppression, physiological stress, and environmental stressors all together impact the overall fitness and survival of pinnipeds.

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CHAPTER 5

DISCUSSION

A current concern in marine ecosystems is the health effects associated with the exposure of organisms to a complex mixture of toxic contaminants, especially those species at higher trophic levels. Marine mammals occur in almost all marine habitats making them good sentinel species for understanding the hazard of these contaminants (Bossart, 2011; Moore, 2008). According to the IUCN Red List, 11 of the 34 species of pinnipeds evaluated are at risk of declining population sizes with seven being listed as endangered (IUCN, 2020). Pollution and/or disease are considered important factors as listed threats for 31 pinniped species (IUCN, 2020). Studies have shown that increased accumulation of toxic contaminants can affect immune system function, which in turn could increase susceptibility to disease (De Swart et al., 1996; Desforges et al., 2016; Mos et al., 2006; Ross, 2002; Van Loveren et al., 2000). For effective management and conservation of pinniped species, it is important to understand the potential hazard these persistent and bioaccumulative contaminants pose. In this dissertation, I demonstrate that both legacy and emerging persistent organic pollutants (POPs) are accumulating in the blubber of wild pinnipeds in multiple locations across the northern hemisphere at levels that have the potential to cause toxic effects for all age classes and sexes.

5.1 Implications for Stranded Seals

The last decade has seen an increase in the number of seals stranding along coastlines around the world. In the US, there are two active unusual mortality events (UME) for pinnipeds with one being attributed to disease. The other is of undetermined cause (OPR, 2020). An increase in stranded seals is often the first sign of a problem that can lead to a UME.

Dehydration, disease, and injury are all factors that can lead to strandings; however, it is unclear why there is an increase in global stranding numbers. Climate change, human interaction, and contaminants may all be contributing factors (Jepson et al., 1999; Jepson et al., 2005; Soulen et al., 2013).

Stranded animals are often considered immune compromised which could be related to contaminant-induced impacts on the animals' immune system. Young animals have been shown to strand more often than other age classes (Soulen et al., 2013; Soulen et al., 2018). For young seals, the immune system is still developing meaning that susceptibility to disease is likely higher compared to older individuals (Frouin et al., 2010; Frouin et al., 2011). Contaminant burdens coupled with other physiological stressors and immune suppression may also play a role in explaining increased stranding numbers. The concentrations of PBDEs measured in stranded seals in this study fall above toxicity reference values estimated in this dissertation. This is even more important considering that all but a very few of those seals were young animals going through important stages of growth and development. Greig et al. (2011) found that stranded harbor seal pups that nursed in the wild and rapidly lost weight during post weaning had the highest contaminant concentrations compared to those that were stranded as neonates (Greig et al., 2011). Other studies examining contaminant concentrations in stranded pinnipeds found very high concentrations of contaminants in all ages classes, including pups and yearlings (Blasius and Goodmanlowe, 2008; Greig et al., 2011; Kajiwara et al., 2001). The high PCB concentrations in stranded pinnipeds from California were the main driver of the overall high blubber ΣPCBs this study found. In some cases, the concentrations in these stranded pinnipeds exceeded the estimated TRVs by up to 10 times. This vulnerability to

effects of contaminants is likely due to recirculation of compounds during fasting and could be a contributing factor to increased numbers of stranded young seals (Greig et al., 2011).

5.2 Implications for Pinnipeds

All pinnipeds are reliant on blubber stores for thermoregulation and as a source of energy during different periods of their life history (O'Hara and O-Shea, 2001; Riedman, 1990). This reliance on blubber as an energy source can lead to remobilization of stored contaminants allowing them to be metabolized or to interact with cellular processes that could affect health, immune function, and eventually overall fitness (De Swart et al., 1996; O'Hara and O-Shea, 2001; Wolkers et al., 2006). The amount of lipids stored in blubber can change rapidly throughout the year causing contaminants to be remobilized but the rate at which this occurs and the effects on the toxicological impacts of the contaminants is not well understood (O'Hara and O-Shea, 2001). This study found that in northern fur seals, increased contaminant concentrations were correlated with increases in the expression of genes involved in contaminant metabolism pathways. These metabolism pathways work through the induction of enzymes which are involved in the metabolism of not only contaminants but also endogenous compounds like hormones. Alterations in the metabolism of hormones can have an indirect effect on the downstream pathways that those hormones control including blubber metabolism, growth, and development.

Pups and yearlings, especially those found in Artic and sub-Arctic regions, rely on blubber for energy while learning how to forage and transitioning to more typical prey species (Lavigne and Kovacs, 1998; Riedman, 1990). Thyroid hormones and the cellular pathways they regulate are important for growth and development, but also the maintenance and function of

blubber. The present study found that contaminants in blubber of northern fur seals were positively correlated with increased expression of TR-α, which could impact the formation of proteins that are required for the metabolism of blubber during a crucial period. Recirculation of contaminant loads during this period could also potentially affect growth and development (Addison and Stobo, 1993; Frouin et al., 2011; Villanger et al., 2013). The release of contaminants themselves from blubber may not always pose a high risk, but metabolites that can be formed from those parent compounds have the potential to have a greater effect. The estimated TRV for toxic effects on thyroid hormone circulation and PCBs was ~15,000 ng/g, but when looking at the TRV for thyroid hormone effects and OH-PCB metabolites, they were significantly lower, 4.66 ng/g and 1.01 ng/g. These lower TRVs indicate that effects may be occurring at much lower concentrations and that the parent compounds may not always be the source of the risk.

Studies examining the intersection between contaminant concentrations and disease or immune suppression are important due to increased numbers of disease outbreaks in pinnipeds (Hutchinson and Simmonds, 1994; O'Shea, 1999; OPR, 2020). This study estimated TRVs for immune effects and PCBs to be 2,358 ng/g and for PBDEs 113.89 ng/g. Though no Northern fur seal had ΣPCB or ΣPBDE concentrations that exceeded the TRV for the different contaminant groups, the ΣPOPs exceeded the PBDE TRV for all seals and a few seals (3 of 16) exceeded the PCB TRV. A study on harbor porpoises in the United Kingdom found that individuals that died of infectious disease had significantly higher PCB loads than those that died due to physical trauma independent of other demographic factors (Jepson et al., 2005). When comparing to the proposed threshold for adverse health effects in marine mammals, 17,000 ng/g lw,

individuals who died of disease more often fell above the threshold than those that died of other causes (Jepson et al., 2005). This potentially indicates a causal relationship between PCB exposure and disease (Jepson et al., 2005). A recent study in California showed a link between elevated PCB concentrations and herpes virus infection in association with elevated cancer prevalence in California sea lions (Gulland et al., 2020). Impacts of contaminant-induced suppression of the immune system may be indirect by acting through the modulation of how the immune system responds and the effectiveness of that response (Gulland et al., 2020). Understanding the impacts of complex mixture of contaminants on the function of physiological systems may be just as crucial as looking at individual groups when assessing risk.

Pinnipeds are exposed to multiple stressors, both natural and anthropogenic, that can interact to affect global pinniped populations. This study demonstrates that even though most persistent organic pollutants (POPs) are no longer in production, they are still found at concentrations that can cause adverse health effects. Concentrations of POPs in many parts of the world have decreased over the last few decades, but continued input from atmospheric circulation, degradation of products, and point sources keep the contaminant burdens in species like pinnipeds higher. There are also inputs of new contaminants that have either unknown toxicity or have been little studied. This complex mixture of contaminants can lead to adverse health issues that are compounded by other stressors. Within the northern hemisphere, a number of pinniped species are reliant on seasonal ice cover for pupping, breeding, and molting (Johnston et al., 2012; Lavigne and Kovacs, 1998). Years where the available ice cover is decreased, those seals are forced to find other areas to use that can lead to overcrowding and easy spread of disease (Johnston et al., 2012; Lavigne and Kovacs, 1998).

Decreased ice cover can also force young seals into the water before they are old enough to forage for long periods (Johnston et al., 2012; Lavigne and Kovacs, 1998). Seals exposed to high concentrations of contaminants at a young age might not be able to handle these environmental changes as easily due to suppression of both immune and endocrine functions. For some pinniped species that are in recovery from previous exploitation, exposure to these contaminants could impact reproduction, survival, and overall growth of the populations.

5.3 Marine Mammals as Sentinel Species of Ecosystem Health

Sentinel species are those that can be used to detect changes in an ecosystem before the effects of those changes become irreversible (Reddy et al., 2001). When studying aquatic systems, oceans are excellent at facilitating the distribution of toxic chemicals (Reddy et al., 2001). It is becoming increasingly important to have good sentinel species for ocean health and to understand how changes in those species can be used as early warning signs of bigger problems or how human health could be impacted by ocean systems in distress.

Marine mammals are good sentinel species of ocean health for a variety of reasons. They are long-lived, high trophic level feeders that rely on large fat stores for a source of energy, which would make them good indicators of mid to long term environmental change. Marine mammals occur in almost all marine environments and some freshwater systems. This factor is crucial because some species have adapted to inhabit a wide range of marine habitats, allowing for the impact of a stressor to be studied across multiple systems in one organism. Marine mammals are vulnerable to similar contaminants as humans and in some instances share a food source/water body. Marine mammals may also be a food source for some populations of people (Bossart, 2011; Moore, 2008). They are highly charismatic species that

humans care about which is important in choosing a sentinel species (Reddy et al., 2001).

A major concern with ocean health is the health effects associated with the complex mixture of toxic contaminants. Studies done using marine mammals could help researchers to start to understand the synergistic effects of these mixtures in a long-lived, high trophic level organisms that could be indicative of the impact of these mixture on human health (Bossart, 2011; Ross, 2000). Marine mammals long life span allows for temporal studies of contaminants which can help to understand how contaminant exposure patterns are changing and which areas are still of high concern. Due to their high trophic level, marine mammals can be monitored to indicate the emergence of diseases or the outbreak of ocean phenomenon like harmful algal blooms that could be a threat to human health (Bossart, 2011; Moore, 2008). An increase in disease frequency tied to contaminant accumulation observed in marine mammals could indicate increasing environmental pressure on those species which in turn can be indicative of environmental distress syndrome (Epstein, 1997).

5.4 Future Research Directions

Understanding the effects of exposure to complex mixtures of contaminants in conjunction with co-exposure to other stressors will be crucial in pinniped research moving forward. Both field and lab studies focusing on contaminant mixtures will aid in the understanding of what threshold levels for effects are as well as how toxicity of compounds differs in the presence of other compounds or stressors. Furthermore when possible, determining accumulation across multiple age classes since contaminant accumulation likely varies with age and life history. Future studies focusing on the relationship between circulating and stored contaminants, especially during times of fasting, will be helpful in knowing the effect

on metabolism pathways and if some effects vary over the course of the year, i.e. thyroid hormone levels being more or less effected. In light of recent evidence, collaborations between contaminant research and disease research may help in better understanding increases in disease emergence in pinnipeds and finding more direct causal effects.

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APPENDIX A

SUPPLEMENTARY TABLES FOR CHAPTER 2-3

Table 2.S1: Mean concentration by major congener group (±1SD) in ng\g ww for harp and hooded seals, by sex and by condition code. For sex and condition code, any individual with unknown was excluded. *-denotes significance between species

	Harp Seals	Hooded Seals	Female	Male	Code 1	Code 3
Tetra-BDE (47)	36.43 ± 16.03	36.90 ± 18.04	32.85 ± 14.48	41.45 ± 19.55	50.02 ± 36.65	34.15 ± 19.82
Penta-BDE (99,100)	12.04 ± 6.75	22.37 ± 16.75*	16.46 ± 13.51	15.14 ± 11.34	19.27 ± 12.01	11.70 ± 11.69
Hexa-BDE (153,154)	8.97 ± 4.76	10.34 ± 4.25	7.47 ± 5.18	7.85 ± 6.58	7.72 ± 6.25	6.44 ± 4.08

Table 2.S2: Stranding Condition Codes

Code	Description
1	Alive
2	Fresh Dead
3	Moderate Decomposition
4	Advanced Decomposition
5	Mummified/skeleton
6	Unknown

Table 3.S1: List of all chemicals analyzed in norther fur seal blubber, quantitation peak (target and transition ions), and the minimum detection limits.

Chemical	Quantitation Peak	MDL (ng/g)
BDE 47	326/217	1.91
BDE 99	297/137	1.94
BDE 100	406/297	2.15
BDE 153	143/62	1.10
BDE 154	141/62	2.17
2 MeOBDE 68	356/313	1.68
6 MeOBDE 47	204/125	2.90
PCB 18	256/186	1.60
PCB 28/31	256/186	2.40
PCB 20	256/186	1.17
PCB 52	290/220	2.97
PCB 44	290/220	1.25

Chemical	Quantitation Peak	MDL (ng/g)
PCB 101	326/256	1.31
PCB 149	360/290	1.87
PCB 118	324/254	1.17
PCB 153	360/290	1.14
PCB 105	324/254	1.43
PCB 138	360/290	1.18
PCB 180	394/324	1.20
PCB 170	394/324	1.79
PCB 194	428/358	1.32
Alpha BHC	181/145	2.52
Beta BHC	181/145	2.30
Gamma BHC	181/145	2.33
Delta BHC	181/145	1.74
Heptachlor epoxide	353/263	0.78
Alpha chlordane	375/266	1.36
DDE	246/176	2.32
Dieldrin	277/241	1.97
DDD	165/115	84.10
DDT	237/165	3.69